CEREAL CHEMISTRY

VOL. XI SEPTEMBER, 1934 NO. 5

STUDIES ON EXPERIMENTAL BAKING TESTS. I. EFFECTS OF VARIATION IN BAKING FORMULAS ON GAS PRODUCTION AND LOAF VOLUME [1, 2]

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(Presented at the Annual Meeting, June, 1934)

Introduction

The baking properties of wheat flour doughs have been shown to be attributable to two distinct sets of factors, those of gas production and those of gas retention. These two sets of factors have received particular attention from the standpoint of their relationship to "fermentation tolerance." Various investigators have placed emphasis on either gas production or gas retention as being of primary importance in considerations of fermentation tolerance. Wood (1907) showed that the size of the baked loaf could be positively correlated with the total gas produced by a fermenting flour suspension. Baker and Hulton (1908), on the other hand, in their study on diastase in flour and gas production capacity, pointed out that while "baker's marks" may increase with gas produced, weak flour did not show this relationship. They concluded that the gas retaining power of the dough was the more important. Martin (1920) showed that the size of the loaf was not correlated with the gas produced in doughs over a 24 hour period, but that improved loaves were obtained with increase in gluten content. Humphries and Simpson (1909) have emphasized that gas production properties should be isolated from gas retention properties in consideration of baking strength. Reference to his work has been made by Fisher and Halton (1932), who state that "the gassing power of flour is independent of its strength and should be regarded as a separate problem from

[1] Subcommittee report, 1933–34 Committee on the Standardization of Laboratory Baking.

[2] Issued as paper No. 52 of the Associate Committee on Grain Research, National Research Council of Canada, with financial assistance from the Saskatchewan Agricultural Research Foundation and the National Research Council of Canada. Condensed from a thesis filed at the Department of Chemistry, University of Saskatchewan, in partial fulfillment for the degree of Master of Science, 1932.

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strength." This view is upheld by Jørgensen (1931). Blish and Sandstedt (1927) have shown that gas production is in no way related to gas retention. Many workers have concluded that the capacity of flours to produce gas when made into doughs is not so important as the ability to retain the gas, because gas production is more amenable to control.

While gas production and gas retention are effects of different causes and therefore ought to be considered mutually independent, they both, however, contribute to the baking characteristics of flour and from this point of view should always be considered together. In other words, either one of these factors may be a limiting factor in the baking test. The combined effects of these two factors are responsible for the "fermentation tolerance" of doughs, a term widely used, but not exactly defined. Swanson and Kroeker (1932) describe it as "the range in the time of fermentation under which a flour will make good bread." Obviously gas production and gas retention are involved, since the time range of tolerance to fermentation is a function of both. These workers indicate also that the harmful effects of over-fermentation are due to sugar exhaustion together with physical and chemical modification of the gluten. Blish and Sandstedt (1927), however, point out that prolonged fermentation does not materially affect the gluten and therefore does not affect the gas retention properties. This is true, provided that the gluten present is of such quantity and quality as to maintain its gas retaining properties against the effects of acids and other products of fermentation. Blish and Hughes (1932) arrive at the conclusion that the products of fermentation have very little influence on gluten or gas retaining potentialities, as a result of work done on a standard commercial patent under ordinary baking procedure. They also conclude that fermentation tolerance "depends predominantly —and for all practical purposes exclusively—upon maintained gas production, as governed by the supply of fermentable sugar."

Gas production depends on the enzymatic activity and carbohydrate content of the dough, while gas retention depends on the physical properties of the gluten. Thus, in making comparative baking tests of flours, the strength may be said to be a function of the gluten, provided gas production is adequate and uniform for each flour throughout the test. A flour may have sufficient gluten strength to give a good loaf but may be unable to do so on account of lack of adequate gas production in a fixed straight dough procedure. A fixed procedure with this limitation may lead to a wrong estimate regarding the baking quality of the flour, as was pointed out by Jørgensen (1931) in his criticism of the A. A. C. C. baking test. In order to show the baking potentialities of any flour the diastatic activity or whatever factors are responsible for

gas production in the later stages of fermentation may have to be supplemented. This is particularly necessary for experimentally milled flours, since it is well known that these are generally markedly lower in diastatic activity than commercially milled flours. Gas production being under control, the strength of the flour then depends on the gluten which is mainly responsible for gas retention properties. The inherent differences of various types of wheat are due mainly to the quantity and quality of the gluten. On this will depend the strength of the wheat when milled into flour, made into dough and baked, provided gas production is adequate. Thus the limiting factor for weak flours is the gas retention, while for strong flours it is the gas production; in other words, *the time range of fermentation is limited by gas retention in weak flours and by gas production in strong flours.* Since in the bread baking industry usually only strong flours (*i.e.*, those of adequate quality and quantity of gluten) are dealt with, then the gas production factor becomes the more important.

It is necessary here to consider some of the factors, other than those involved in the flour itself, that may affect gas production. Chief among these are mechanical treatment, yeast variability and chemical improvers. Working (1929) has discussed some mechanical effects of punching and products of fermentation without arriving at any very definite conclusions. Fisher and Halton (1932) show that "knocking back" tends to increase the rate of gas production after a period of three hours. The rate then progressively increases with the number of punches, according to the data presented, until the sixth hour is reached, when the values begin to converge. The maximum differentiation of rate of gas production with punching appears to be at the fifth hour. The authors attempt no explanation of this, but simply state that "knocking back" has a marked effect on gas production. Dunlop (1932) observed that punching increases gas production slightly, but concluded that the effect was negligible from the baking standpoint.

Of the various chemical improvers, only potassium bromate was used in this study. The effect of this salt on baking results has been discussed in some detail by Geddes and Larmour (1933) and need not be recapitulated here. Discussion of the effect of potassium bromate on gas retention of doughs will be made when considering the experimental data.

With respect to the variability of yeast, it is the experience of this laboratory that shipments received week by week do not change very markedly as shown by baking tests. The particular brand of yeast used did not change with regard to gas production potentialities when stored on ice over a period of 7 weeks as shown by Larmour and Brockington (1932). Toward the end of this period, loaf volumes from doughs

baked with this yeast became erratic, but generally tended to increase. Weaver, Talbott, and Coleman (1933) reported considerable change in loaf volume during the course of 168 hours with one of two yeasts examined. The other, however, produced little change in loaf volume. In the following work fresh yeast was always used; gas production measurements and the corresponding baking data were always obtained with yeast from the same cake.

While numerous studies on the effect of variation in baking procedure have been made, little information is available regarding the effect of varying the baking formula in regard to sugar and yeast content. The work reported in this paper represents an attempt to go more thoroughly into the problem of gas production in dough and the relationship of this to loaf volume, and to this end it was decided to ascertain the effects of different formulas and procedures on both total CO_2 production and rate or intensity of CO_2 production, during various times in the fermentation period.

Experimental

The apparatus used in measuring gas production and retention was similar to that of Bailey and Johnson (1924). The gas was measured in an inverted burette suspended in a tube containing concentrated NaCl solution. A modification of the basic A. A. C. C. formula was used in making up the doughs, and consisted of 100 gms. of flour (13.5% moisture basis), 3 gms. yeast, 2.5 gms. sugar, 1 gm. salt, with added water to give the dough the proper consistency. When it was desired to study the effect of addition of potassium bromate, 0.001 gm. was added to the ingredients. After mixing the dough was aliquoted into four parts, duplicate doughs being placed in beakers surrounded by either 20% KOH or 20% NaCl contained in jars of an appropriate size. These were placed in a water thermostat maintained at 30° C. Readings were taken every ten minutes. For doughs over KOH, wherein volumes attained by the doughs were measured, readings were discontinued at the first collapse of the dough, as measurements beyond this point became somewhat erratic.

Effect of Punching and of $KBrO_3$ on CO_2 Production and Retention

For this study the first dough designated as " unpunched " was taken directly from the mixer. A second dough was fermented 105 minutes to the first punch and was then placed in the apparatus; this was designated as the " first punch " dough. A third dough was given the first punch, fermented 50 minutes longer to the second punch and then placed in the apparatus; this was designated as the " second punch " dough. A fourth dough was given the first and second punches, fer-

mented an additional 25 minutes, punched down and placed in the apparatus; this was designated as the " third punch " dough. These times correspond to the regular baking procedure of the A. A. C. C., and afford a method for estimating the effect of punching or of previous fermentation on the properties of the dough. Both basic and bromate formulas were used. A description of the flours used in this study is given in Table I. Determinations of CO_2 production and retention were made by this method on a number of different flours, but, as it would be impossible in a paper such as this to present all the data, we shall consider only three typical cases, namely, flours Nos. 1, 2, and 3, described in Table I.

TABLE I

ANALYTICAL DATA ON FLOURS USED IN THIS STUDY

No.	Description	Per cent protein (13.5% moisture basis)	Per cent ash (13.5% moisture basis)	Diastatic activity (Rumsey units)
1	Canadian hard red spring, Commercial patent	12.1	0.41	163.0
2	Pacific Club, commercial patent	7.0	—	—
3	Canadian Marquis, experimentally milled. Crop of 1929	17.7	—	—
4	Canadian Marquis, high protein experimentally milled composite	16.5	0.63	93.0
5	Canadian Marquis, low protein experimentally milled composite	10.2	0.72	169.5

Considering first gas retention, the results obtained with flour No. 1, as shown graphically in Figure 1, indicate that punching increased gas retention with both the basic and bromate formulas. There was no differentiation attributable to bromate in the unpunched and the first punch doughs, but in the second and third punch doughs the bromate caused an increase in gas retention, so that the effect of punching appeared greater with the bromate formula than with the basic formula.

With flour No. 2, the club wheat patent, punching gave an increase of about 12% in CO_2 retention with the basic formula, but no increase whatsoever with the bromate formula. The CO_2 retention was less with the bromate than with the basic formula in each of the four trials, the difference being greater with " third punch " dough than with the unpunched dough.

The data for flour No. 3, shown graphically in Figure 3, are quite different from either of the preceding cases. The first punch resulted in increased CO_2 retention with both basic and bromate formulas, but the second and third punch doughs were lower in volume. Apparently

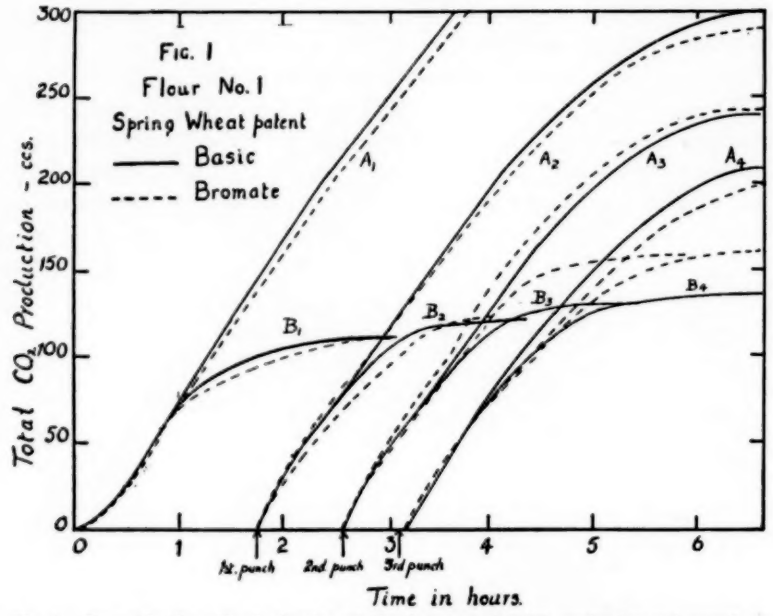

Fig. 1. Curves for flour No. 1, showing relation between total CO_2 production and retention with punching, 0.001% $KBrO_3$ and fermentation time on aliquot of dough representing 25 gms. of flour. Curves marked "*A*" show total CO_2 production and those marked "*B*" volume attained by the dough. A_1 and B_1 are for doughs fermented immediately after mixing, A_2 and B_2 after first punch (105 mins.), A_3 and B_3 after second punch (155 mins.), A_4 and B_4 after third punch (180 mins.).

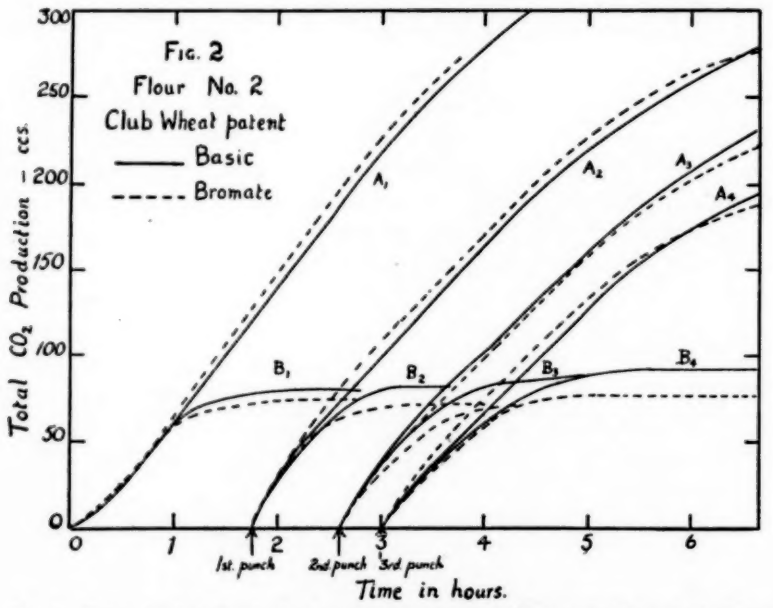

Fig. 2. Curves for flour No. 2, showing relation between total CO_2 production and retention with punching, 0.001% $KBrO_3$ and fermentation time on aliquot of dough representing 25 gms. of flour. Curves marked "*A*" show total CO_2 production and those marked "*B*" volume attained by the dough. A_1 and B_1 are for doughs fermented immediately after mixing, A_2 and B_2 after first punch (105 mins.), A_3 and B_3 after second punch (155 mins.), A_4 and B_4 after third punch (180 mins.).

the CO_2 retention of the dough reached a distinct maximum in the period succeeding the first punch and then fell off quite markedly. This evidently is not attributable to weakness of the gluten, because in all four trials the CO_2 retention by the bromate formula was equal to or greater than that for the basic formula, which is different from the case of the weak club patent.

An explanation for the decreased gas retention in flour No. 3, may be found in the sharp falling off of CO_2 production as shown by the *A*

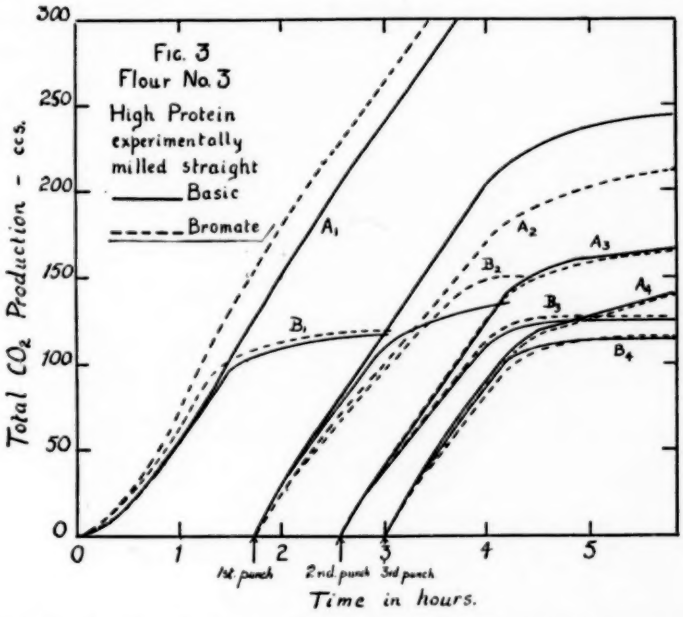

Fig. 3. Curves for flour No. 3, showing relation between total CO_2 production and retention with punching, 0.001% $KBrO_3$ and fermentation time on aliquot of dough representing 25 gms. of flour. Curves marked "*A*" show total CO_2 production and those marked "*B*" volume attained by the dough. A_1 and B_1 are for doughs fermented immediately after mixing, A_2 and B_2 after first punch (105 mins.), A_3 and B_3 after second punch (155 mins.), A_4 and B_4 after third punch (180 mins.).

curves in Figure 3. Comparing the CO_2 production curves of Figure 3 with those of Figures 1 and 2, it can be seen that in the former there was a very decided drop in the total gas produced in the second punch doughs, so that one hour and 35 minutes after the dough was placed in the apparatus, the CO_2 production had practically ceased. With the third punch dough of flour No. 3, CO_2 production had reached a very low rate in about the same time and the total quantity produced in this case was still less than in the second punch dough. Consequently, no matter what may have been the CO_2 retention potentialities of these doughs, they were unable to expand on account of lack of gas. It

should be noted that when CO_2 production became a limiting factor, differentiation by bromate, in respect to dough volume, disappeared.

Considering next the total CO_2 production, it can be seen in Figures 1 and 2 that the effect of punching was apparently negligible with both the basic and bromate formulas in the cases of flours 1 and 2. If punching had had any considerable influence on CO_2 production, it would have been manifested by a pronounced change in slope of the "A" curve corresponding to the various punches. There was no significant differentiation attributable to bromate in respect to CO_2 production in these doughs. Flour No. 3 apparently presents an exception inasmuch as the first punch dough seems to have produced less CO_2 by the bromate than by the basic formula. Some doubt is thrown on the

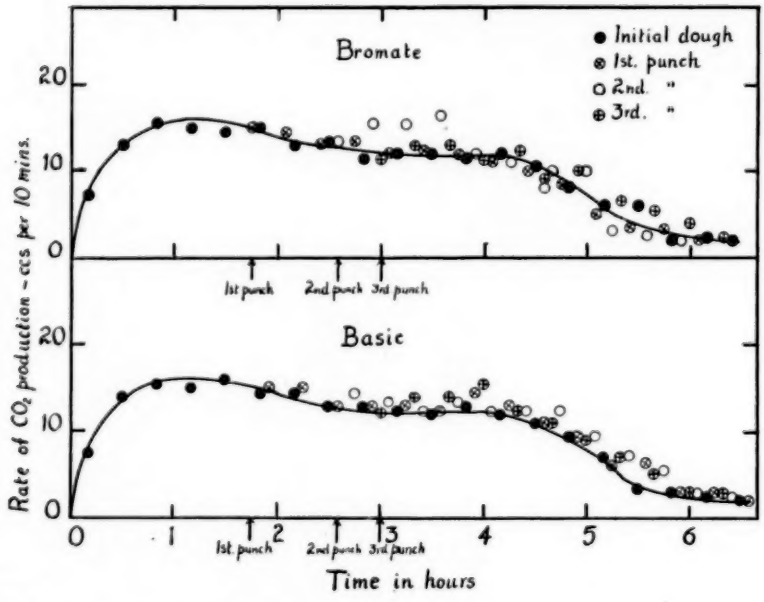

Fig. 4 (*a*). Curves showing effect of punching and $KBrO_3$ on rate of CO_2 production for flour No. 1. Rates are shown for doughs taken immediately after mixing, after the first punch (105 mins.), after the second punch (155 mins.), and after the third punch (180 mins.).

validity of this observation by the fact that there was no similar differentiation in the second and third punch doughs.

These facts can be seen much more clearly by a study of the rate curves for CO_2 production as shown in Figure 4, in which the volume of CO_2 produced in ten minute intervals is plotted against time. It can be seen that the rates for the different punches are nearly coincidental with the curve for the initial dough, except in the case of flour No. 3 in which it appears that punching had the effect of maintaining the high

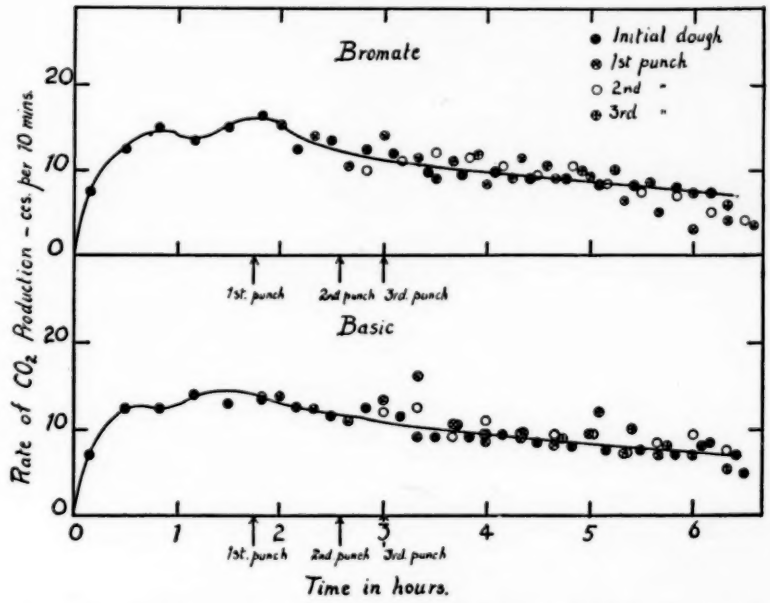

Fig. 4 (*b*). Curves showing effect of punching and $KBrO_3$ on rate of CO_2 production for flour No. 2. Rates are shown for doughs taken immediately after mixing, after the first punch (105 mins.), after the second punch (155 mins.), and after the third punch (180 mins.).

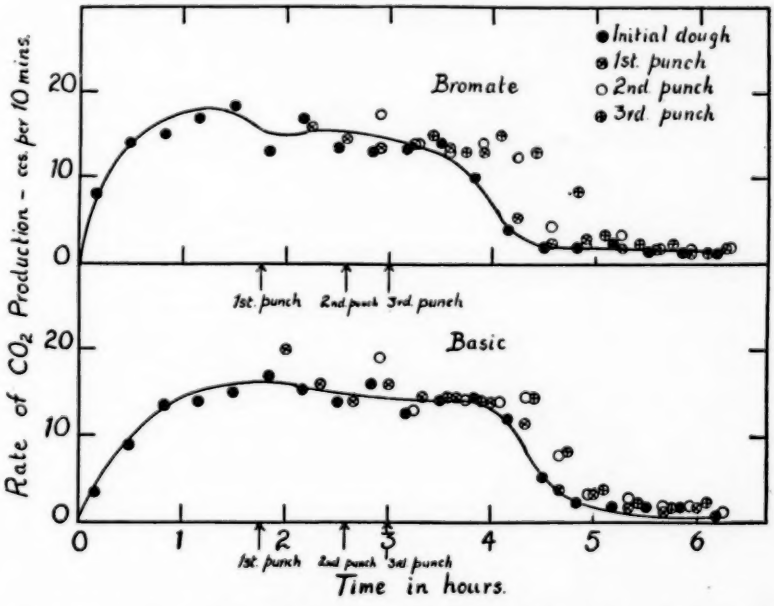

Fig. 4 (*c*). Curves showing effect of punching and $KBrO_3$ on rate of CO_2 production for flour No. 3. Rates are shown for doughs taken immediately after mixing, after the first punch (105 mins.), after the second punch (155 mins.), and after the third punch (180 mins.).

gassing rate for a longer period. Thus in the initial doughs by the basic formula the rate started dropping sharply at about four hours, whereas the third punch doughs did not show a corresponding drop until four and one-half hours. This difference appeared to be accentuated by the bromate because these doughs started decreasing at about 3.5 hours, but it should be noted that both second and third punch doughs started decreasing in rate at the same time as in the corresponding basic doughs. There is therefore no reason to conclude that there was a differential effect attributable to bromate. There does seem to have been a depressing effect on rate of CO_2 production in the initial and first punch doughs. This depressing effect of bromate on CO_2 production in initial doughs has not yet been confirmed.

Summarizing the foregoing discussion it may be noted that punching tends to increase gas retention in strong flours and has no effect on a weak flour, and that it has little, if any, effect on the rate of gas production in doughs made up by either the basic or bromate formula.

Potassium bromate has a distinct effect on gas retention of doughs, increasing it in the case of strong flours and decreasing it in the case of the weak flour when gas production is adequate.

Relation of Dough Formula to Rate of CO_2 Production

It was pointed out in the introduction that the time range of fermentation of strong flours may be limited by gas production. It is important, therefore, in any study of baking formulas to determine precisely the time range over which the dough will produce sufficient CO_2 for adequate expansion, particularly in the pan-proof period. For this purpose the rate of CO_2 production is a more useful expression of the facts than the total CO_2 production, and, therefore, this method of presenting the data has been used in our work.

In order to investigate the effect of changes in percentage of yeast and sugar in the baking formula, flours Nos. 1, 4, and 5, listed in Table I, were used. With each of the first two flours twelve modifications of formula were used in determining the rate curves for CO_2 production, while with flour No. 5 only nine were employed on account of shortage of material. The data obtained are presented graphically in Figure 5. The figure contains three sets of curves corresponding to the three flours studied, each set showing four groups of curves representing 0%, 1%, 2.5%, and 6% added sugar. In each group, except in the case of flour No. 5, there are three curves representing 1%, 3%, and 5% yeast, all percentages being based on flour at 13.5% moisture content. In all cases the data were obtained on initial doughs in duplicate, taken di-

rectly from the mixer and fermented in the gas measurement apparatus without punching, usually for a period of slightly over six hours.

The simplest way to consider the data presented in Figure 5 is to divide the discussion of the formulas into three parts on the basis of yeast content. Looking first at the curves for 1% yeast, it can be seen that they are all practically the same except in the case of the no-sugar

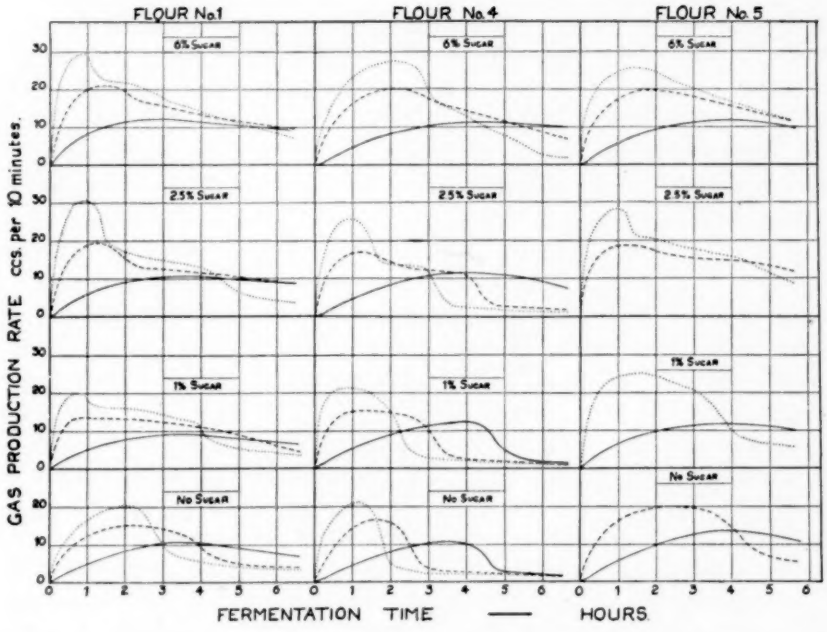

Fig. 5. Comparative rates of CO_2 production in doughs fermented with varying amounts of sugar and yeast, for flours Nos. 1, 4, and 5. ———— 1% yeast – – – – – 3% yeast ····· 5% yeast.

and 1% sugar levels of flour 4. This means that with flours 1 and 5, the yeast was the limiting factor and that the diastatic activity was sufficiently high to provide enough sugar to allow the yeast to work at its maximum rate throughout the period of the experiment, this rate being fairly close to 10 cc. CO_2 per 10 minutes. It should be noted that the rates for all sugar levels increase for the first two hours and then become fairly constant until about the fifth hour when there appears a tendency to decrease. This gradual decline from the maximum rate (attained between the second and fourth hours) occurs at all the sugar levels and seems to be due to decrease in yeast activity.

Flour No. 4, which was low in diastatic activity, provides exceptions in the no-sugar and 1% sugar levels, where the rate of CO_2 production decreased abruptly before the end of the observation period. This must

be interpreted as due to sugar exhaustion. In the case of the "no-sugar" formula, the initial sugar in the flour plus that produced by diastatic action was sufficient to maintain the gas rate at its maximum for four hours; after that, the yeast, having used up the surplus, had to depend on sugar produced by enzymatic action and on account of the low diastase there was not sufficient to maintain the gas rate. Diastatic activity of the flour, therefore, became the limiting factor in gas production after the fourth hour. The addition of 1% sugar to the formula maintained the maximum gas rate for an extra half hour. With addition of 2.5% sugar, however, the curve was similar to those of the other flours.

With 3% yeast, a great many irregularities in the rate curves were observed and therefore it is not possible to make a very rigid comparison between sugar levels and between flours in respect to maximum rates and times of attaining them. Maximum rates, however, are likely much less important than minimum rates, because, no matter what the rate of gassing in the early part of the fermentation period, it is essential to have a certain sustained rate of gas production during pan-proofing. No matter how well developed the dough may be, it cannot make good bread if there is not sufficient CO_2 produced to provide for rising in the pan. We have concluded that the minimum rate for proper proofing is about 10 cc. per 10 minutes for 25 gm. formula. This, of course, is bound to vary with the gas retention property of the dough, but good loaves have seldom been obtained when the rate was much below this value, and this will be referred to as the "minimum adequate gas rate."

With flour No. 1 and the no-sugar 3% yeast formula, sugar exhaustion became noticeable at about three and a half hours. The effect of 1% sugar was to maintain the minimum adequate gas rate one hour longer. With 2.5% sugar, it was maintained for five and a half hours, or two hours longer than with the sugarless dough. Further addition of sugar failed to increase this time range. With flour No. 5, similar results were obtained.

Flour No. 4 shows very clearly the effect of low diastatic activity and the results of additions of sugar. With the no-sugar 3% yeast formula, the gas rate started to decrease sharply at about 2 hours and at 3 hours was down to the same rate as with the 1% yeast. Addition of 1% sugar postponed the drop to two and a half hours; 2.5% sugar postponed it to nearly four hours, while with 6% sugar it did not fall below the minimum adequate rate until five and a half hours.

As 3% is the amount of yeast prescribed for the standard baking formula, it is interesting to note the probable effects of applying the standard fermentation times to low diastatic flours. The proofing be-

gins after the third hour and lasts for 55 minutes. If the gas rate is falling during this period, there likely will be insufficient CO_2 for proofing. It can be seen that even with 2.5% sugar the rate would be decreasing slightly in the latter part of the proof period. The regular fermentation period, therefore, would barely provide the proper conditions for such flours. Obviously any attempt to study the strength factor in fermentation tolerance by extending the fermentation time would give useless results with this formula. Furthermore, it seems reasonable to predict that flours of lower diastatic activity would be badly misjudged by application of this particular formula. For this type of flour either of three modifications of procedure are indicated—(1) the fermentation time should be reduced so as to bring the proofing within the period of adequate gas production; (2) the diastatic activity of the flour should be supplemented; or (3) the amount of sugar in the formula should be increased.

With respect to the 5% yeast formulas, the rate of CO_2 production increases more rapidly and attains higher maxima. The effect of this is to exhaust the available sugar sooner and thus make diastatic activity the limiting factor earlier in the fermentation period. The effects of adding sugar are similar to those discussed in the case of the 3% yeast formulas.

Relation of Dough Formula to Loaf Volume

With the foregoing data concerning rates of gas production in hand, we may proceed to study the baking results in relation to the various formulas. Bakings were made in duplicate on the three flours by all twelve modifications of yeast and sugar content, each with and without bromate. The loaf volumes are given in Tables II, III, and IV. These tables also show response to bromate, average rate of CO_2 production during the proofing period and total CO_2 production to the end of the proofing period. The average rate in the proofing period was calculated from the original data, not from the graphs.

Considering first the data for flour No. 1, in Table II, it should be noted that in the 1%, 2.5%, and 6% sugar groups, the loaf volumes for non-bromated doughs increased with increasing yeast content. This does not apply to the no-sugar group because with 5% yeast the rate of CO_2 production dropped to an average value of 6.5 cc. per 10 minutes [5] in the proofing period and this was accompanied by a decrease in loaf volume. This general increase in loaf volume with increasing amounts of yeast might be attributable to increased rate of gassing dur-

[5] In this discussion we shall refer to the gas rates as determined with the 25 gm. formula. Theoretically, for the regular dough, this should be multiplied by 4, but as we do not know whether or not the larger doughs would gas exactly four times as fast as the quarter-size doughs, it seems advisable to use the data actually obtained, keeping in mind the difference in dough size.

TABLE II

LOAF VOLUMES AND CO_2 PRODUCTION BY DIFFERENT FORMULAS USING FLOUR NO. 1—AN AVERAGE HARD RED SPRING COMMERCIAL PATENT

Formula		Loaf volume			CO_2 production data (25 gm. formula)	
No.	Description	Basic	BrO_3	BrO_3 response	Average rate in proof period	Total CO_2 in 240 minutes
		Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*
1a	No sugar + 1% yeast	620	640	+20	10.1	174
1b	" " + 3% "	670	668	−2	11.3	295
1c	" " + 5% "	570	590	+20	6.5	325
2a	1% sugar + 1% yeast	605	620	+15	8.9	155
2b	" " + 3% "	653	660	+7	11.9	298
2c	" " + 5% "	683	655	−28	12.2	353
3a	2.5% sugar + 1% yeast	605	633	+28	10.3	196
3b	" " + 3% "	615	630	+15	10.5	351
3c	" " + 5% "	670	623	−47	13.7	455
4a	6% sugar + 1% yeast	633	660	+27	11.0	229
4b	" " + 3% "	695	658	−37	14.0	394
4c	" " + 5% "	710	625	−85	15.0	485

TABLE III

LOAF VOLUMES AND CO_2 PRODUCTION BY DIFFERENT FORMULAS USING FLOUR NO. 4, AN EXPERIMENTALLY MILLED FLOUR, HIGH IN PROTEIN AND LOW IN DIASTASE

Formula		Loaf volume			CO_2 production data (25 gm. formula)	
No.	Description	Basic	BrO_3	BrO_3 response	Average rate in proof period	Total CO_2 in 240 minutes
		Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*
1a	No sugar + 1% yeast	550	640	+90	10.3	178
1b	" " + 3% "	405	460	+55	3.0	216
1c	" " + 5% "	398	453	+55	2.0	207
2a	1% sugar + 1% yeast	538	720	+182	11.5	195
2b	" " + 3% "	470	620	+150	6.3	249
2c	" " + 5% "	415	520	+105	2.0	243
3a	2.5% sugar + 1% yeast	570	763	+193	10.3	179
3b	" " + 3% "	603	895	+292	12.0	317
3c	" " + 5% "	438	590	+162	4.0	339
4a	6% sugar + 1% yeast	610	845	+235	10.0	174
4b	" " + 3% "	708	1005	+297	15.0	373
4c	" " + 5% "	708	1020	+312	15.0	492

TABLE IV

DATA FROM TABLE III COLLECTED TO SHOW THE SPECIFIC EFFECT OF SUGAR ON LOAF VOLUME AND RESPONSE TO BROMATE

Formulas		Loaf volume			CO_2 production in 25 gm. doughs	
No.	Description	Non-bromated	Bromated	Response to bromate	Average rate in proof period	Total CO_2 in 4 hours
		Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*
1a	No sugar + 1% yeast	550	640	+90	10.3	178
3a	2.5% " + 1% "	570	763	+193	10.3	179
4a	6% " + 1% "	610	845	+235	10.0	174

ing the pan-proof, to dough development resulting from greater amount of CO_2 produced during the fermentation period, or to the action of some specific ingredient of the yeast which would act like a flour improver. The last two possibilities seem the most probable, because the positive effect of bromate is greatest on the doughs with only 1% yeast. The greatest negative bromate differentiations for this flour were observed with formulas having 5% yeast, indicating that these doughs were developed nearly to their maximum by the non-bromated formula.

Another very important observation on this flour is that the effect of bromate on gas retention is dependent on the formula used. As examples of this, formulas 1*b* and 3*c* in Table II may be cited. With formula 1b, no-sugar + 3% yeast, there was 295 cc. CO_2 produced in four hours; the non-bromated and bromated doughs gave practically the same loaf volumes, namely 670 cc. and 668 cc. respectively; gassing in the proof period was above the minimum adequate rate; there was no response to bromate, indicating no change in gas retention due to bromate. With formula 3*c*, 2.5% sugar + 3% yeast, the total CO_2 production was 455 cc. in four hours, indicating a much more intense fermentation; the non-bromated dough gave the same volume—670 cc.—as in formula 1*b*, while the bromated dough gave only 623, a decrease of 47 cc., indicating that the effect of bromate in this formula was to decrease the gas retention of the dough. Again in the case of formula 2*b* there was a total CO_2 production of 298 cc., indicating practically the same intensity of fermentation as with formula 1*b*, and the response to bromate was within the limits of experimental error. Taking total CO_2 production for four hours after the dough is mixed as a measure of the intensity of fermentation, it may be stated that flour No. 1 generally showed positive response to bromate with the lower intensity of fermentation and negative response with the highest in-

tensities. At an intensity of about 300–350 cc. CO_2 per four hours, the doughs gave little or no response.

Flour No. 4, the data for which are given in Table III, presents a quite different case. The response to bromate was positive and of considerable magnitude with all formulas. These data show very well the effect of low gassing rate in the proof period on loaf volume and on response to bromate. With formulas 1*b*, 1*c*, 2*c*, and 3*c* the gas rates in the proofing period were low; loaf volumes were low, and the responses to bromate, while still positive, were lower than they should have been. An excellent example of this can be seen in the results by formulas 2*a*, *b* and *c*, all having 1% sugar 1%, 3%, and 5% yeast respectively. With 1% sugar + 1% yeast, the total CO_2 production was only 195 cc., but the gas rate was 11.5 during the proof period; loaf volume of both non-bromated and bromated doughs was higher than the others in this group; response to bromate also was highest. With formulas 2*b* and 2*c*, the total CO_2 produced was about the same, 249 cc. and 243 cc. respectively, and in both cases higher than with formula 2*a*, indicating a greater intensity of fermentation. But in both cases the gassing rate during the proof period was inadequate; the loaf volumes were lower and the responses to bromate were lower. Again with formulas 3*b* and 3*c* it can be seen that lowering the gas rate in the proofing period results in lower loaf volume and lower response to bromate.

The data in Table IV provide evidence for the conclusion that sugar has a specific effect on the loaf volume of this flour. These particular data were selected because they were obtained with formulas that gave practically the same intensity of fermentation, as indicated by the total CO_2 production, and the same rate in the proof period. They differed only in the amount of sugar added. Loaf volume by both non-bromated and bromated formulas increased with increasing sugar and the response to bromate also showed a marked increase, indicating that there must have been a decided increase in gas retention. This effect can be attributed only to the presence of excess sugar. Similar effects on loaf volume can be seen in the results by formulas 3*b* and 4*b*, Table III. In this case there was no appreciable increase in response to bromate. It is evident from these data that with high protein experimentally milled flour one must be prepared to accept the fact that the amount of free sugar added to the dough may be a limiting factor even when CO_2 production is adequate at all stages of fermentation.

Considering next the low protein experimentally milled flour No. 5, the data for which are given in Table V, it is seen that manipulation of the formula produced relatively little difference in loaf volume. Un-

TABLE V

LOAF VOLUMES AND CO_2 PRODUCTION BY DIFFERENT FORMULAS, USING FLOUR NO. 5, AN EXPERIMENTALLY MILLED FLOUR, LOW IN PROTEIN AND HIGH IN DIASTASE

Formula		Loaf volume			CO_2 production data (25 gm. formula)	
No.	Description	Basic	BrO_3	BrO_3 response	Average rate in proof period	Total CO_2 in 240 minutes
		Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*
1a	No sugar + 1% yeast	550	555	+5	12.8	219
1b	" " + 3% "	560	540	−20	16.7	393
1c	" " + 5% "	488	470	−18	—	—
2a	1% sugar + 1% yeast	573	575	+2	11.5	211
2b	" " + 3% "	573	555	−18	—	—
2c	" " + 5% "	543	515	−28	13.8	475
3a	2.5% sugar + 1% yeast	573	565	−8	—	—
3b	" " + 3% "	580	563	−17	15.0	391
3c	" " + 5% "	538	530	−8	16.3	488
4a	6% sugar + 1% yeast	570	575	−5	12.0	203
4b	" " + 3% "	613	568	−45	16.0	396
4c	" " + 5% "	563	555	−8	17.3	509

fortunately, owing to lack of flour, three of the gas production curves were omitted. It seems probable that the lowering of volume with formula 1*c*, no-sugar + 5% yeast, was due to inadequate CO_2 production during the proof period. In all the eleven other cases, the rates were maintained well above the minimum value of 10 cc. per 10 minutes. What little response there was to bromate was either negligible or negative. This particular flour was singularly unresponsive to the effects of intense fermentation, to large quantities of sugar and to bromate. It is interesting to note that formulas 1*a* and 4*c* with bromate both gave the same loaf volume, despite the fact that the total CO_2 production was 219 cc. and 509 cc. respectively for the four hour period. Comparing all the 1% yeast formulas, namely 1*a*, 2*a*, 3*a*, and 4*a*, very little difference in loaf volume by either non-bromated or bromated formulas was observed.

Leaving aside those formulas which failed to provide adequate gas production at 10 cc. or more per 10 minutes during proofing, it is evident that the greatest differentiation of these three flours was obtained by formula 4*c*, namely 6% sugar and 5% yeast. This applies particularly to the bromated doughs. This formula appears to be the most severe,

as shown by the large negative responses to bromate given by flour No. 1. The dough made with this flour evidently was fully matured by the intense fermentation and could not tolerate the additional effect of bromate. The high protein experimentally milled flour responded to the more intense rate of fermentation, but showed further marked improvement with bromate. It might be concluded, therefore, that this flour possessed greater fermentation tolerance than flour No. 1. Flour No. 5, judged by these standards, would be considered highly tolerant to fermentation, inasmuch as it behaves as well with intense as with mild fermentation, but this conclusion would have to be modified by the observation that it does not behave well with either; it was merely insensitive to varying conditions of fermentation. It should be added here that these high sugar formulas all resulted in heavy overdarkening of crust color and in the case of flour No. 4, the bromated 4*c* formula produced a very open texture. However, in attempting to ascertain strength, it seems to us necessary to get the maximum capacity of the dough for expansion, and therefore only loaf volume has been considered.

From the foregoing discussion it is evident that increasing the intensity of fermentation during a four-hour period ought to give information similar to that obtainable by prolonging the fermentation at a lower average rate, without the risk attendant upon the latter method, namely the reduction of the gassing rate to such a point that there is an inadequate amount of CO_2 for proper proofing.

Even with large amounts of sugar and small amounts of yeast, the rate of CO_2 production tends to fall below 10 cc. per 10 minutes between the fifth and sixth hours. Other experiments have shown that after this period the rate will drop to low values even when there is a good excess of sugar left in the dough. The usual fermentation tolerance procedure by which the doughs are fermented 4, 5, 6 hours longer may bring the dough to the condition in which CO_2 production in the proofing period, rather than gluten strength, becomes the limiting factor. It would appear, therefore, to be more advisable to intensify fermentation by increasing the yeast and sugar in the formula and keeping the time at four hours or less, than to prolong the fermentation with the ordinary formula and run the risk of going short of CO_2 in the proofing. Additional evidence favoring this conclusion is presented in the second paper of this series.

Summary

Gas production and retention measurements were made in a suitable apparatus on typical commercial and experimental wheaten flours of Western Canada. The effect of punching and of $KBrO_3$ on gas pro-

duction and retention of dough was observed. It was found that neither had much effect on gas production, but both had profound influence on gas retention as measured by dough volumes. This followed only when gas production in the dough was adequate in order to attain maximum volume. Changes in gas production and loaf volume with change in dough formula were observed. Studies with experimentally and commercially milled strong and weak flours, using various quantities of sugar and yeast in the dough formulas, have shown that different types of flours respond very differently to variation in fermentation. It was found that loaf volume is closely related to rate of gas production in the proofing period and that bromate response also depended on this factor, except in the case of low protein poor quality flours which generally showed a negative response. Studies of gas rate curves and loaf volumes showed that various estimations of baking strength would be obtained unless limiting conditions of fermentable sugar during proofing are eliminated, particularly with high protein experimentally milled flours.

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STUDIES ON EXPERIMENTAL BAKING TESTS. II. THE APPLICATION OF A HIGH YEAST-SUGAR FORMULA IN EVALUATING FLOUR STRENGTH [1, 2]

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(Presented at the Annual Meeting, June, 1934)

Introduction

In an earlier paper Larmour and Brockington (1934) discussed some of the effects of varying the sugar and yeast in the baking formula on gas production and loaf volume. In that study, the standard fermentation time of 3 hours before panning was used. In this paper we have to report some observations made as a result of applying varying fermentation times to the various formulas hereinafter described.

In testing wheat quality from the export viewpoint, with which we in Western Canada are largely concerned, it appears to be more important to estimate correctly the inherent value of the wheat for blending purposes than to ascertain its value for making flour per se. Many of the wheats tested would make flour altogether too high in protein and too tough in gluten to be used satisfactorily either for domestic or commercial baking. Yet these wheats, or the flour from them, appear admirably suited for blending with softer wheats in order to produce satisfactory commercial flour. It is currently believed that strong wheats and their flours, which by themselves are very intractable to handling, contribute to the blend the capacity to make good bread over a greater time range than the soft wheat alone. Fisher (1933) showed that with soft flours there usually is a very short optimum period for the production of good bread, whereas with blends containing Manitobas, the optimum period is extended considerably.

These and other similar observations point to the conclusion that

[1] Subcommittee report, 1933–34 Committee on the Standardization of Laboratory Baking.

[2] Issued as paper No. 53 of the Associate Committee on Grain Research, National Research Council of Canada, with financial assistance from the Saskatchewan Agricultural Research Foundation and the National Research Council of Canada. Condensed from a thesis presented to the Faculty of Arts and Science, University of Saskatchewan in partial fulfillment for the degree of Master of Science, 1932.

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fermentation tolerance is associated with the character of the flour proteins and should be considered one of the most important of the properties of a strong wheat. Suitable methods for estimating this property of Canadian spring wheats would, therefore, be most useful in attempting the appraisal of their value for export. It seems unlikely, however, that any practical method for direct determination of fermentation tolerance of such wheats will be developed. The best we are likely to achieve is a reliable indication by indirect means.

Fermentation tolerance of a dough depends on two factors, namely, the capacity of the gluten proteins to resist disintegration, and the capacity of the dough to maintain CO_2 production through the proofing period. Blish and Sandstedt (1927) stated that the fermentation can be extended for very long periods without substantially altering the appearance of the bread produced, provided that sugar is added before panning, in order to provide sufficient gas for proving. They concluded that the bad effects of prolonged fermentation can be attributed entirely to lack of sugar in the final stages.

Later studies by Blish and Hughes (1932), Clark (1929), Moen (1930), and Swanson and Kroeker (1932) all indicate that the first emphasis must be placed on maintenance of gas production rather than on the gluten. It would seem, therefore, that fermentation tolerance of American flours depends largely on maintained gas production which can be easily regulated through the dough formula. Nevertheless, the fact remains that average hard red winter or spring wheats in a blend with European soft wheats do increase the fermentation tolerance, although they do not as a rule contribute as much to the maintenance of CO_2 production in the way of diastatic activity, as the soft wheat component. It is obvious that in order to apply the method of extended fermentation time to a study of differences in strong wheats, it is necessary to have formulas that will ensure adequate gas production throughout the time range used.

Experimental

With these considerations in mind, twelve modifications of yeast and sugar, with and without bromate, were applied to two flours, with fermentation times of 2, 3, and 4 hours respectively. Two flours, both experimentally milled composites, were used in this investigation: flour 1 contained 14.7% protein and had a diastatic activity of 108 Rumsey units; flour 2 had 10.2% protein and a diastatic activity of 170 Rumsey units. They represent the extremes which would lead to the greatest error in estimation of relative strength when the basic formula is applied. The problem was to find the formula that would give the best differentiation of these flours, assuming flour 1 to be strong and flour 2 to be weak.

With both flours CO_2 production rates were measured over a period of 5 hours with two formulas—(1) the standard basic and (2) the standard basic + 0.125% diastatic malt (200° Lintner). These rates, expressed in cc. of CO_2 produced per 10 minutes by doughs representing 25 gms. of flour are shown in Figures 1 and 2.

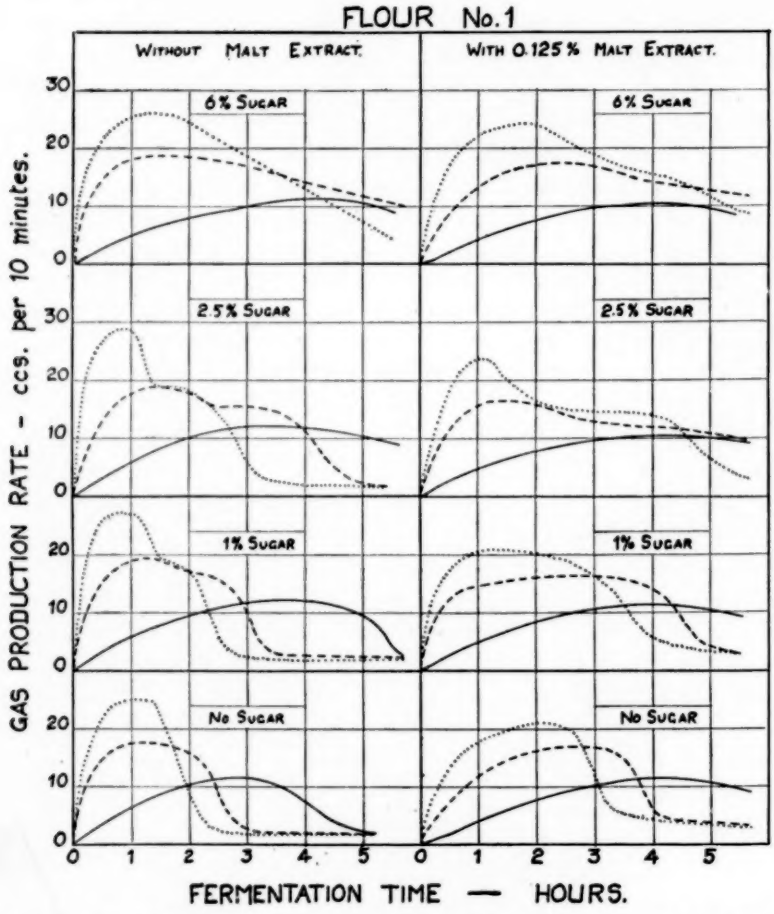

Fig. 1. Curves showing relationship between rate of CO_2 production and variation in baking formula for flour No. 1, with and without malt extract. ——— 1% yeast - - - - - 3% yeast ····· 5% yeast.

Relation of CO_2 Production to Dough Formula

The general relationship between the various formulas used in these studies and their respective gas production rates have already been discussed in the first paper of this series by Larmour and Brockington (1934) and needs no further elaboration here. It should, however, be

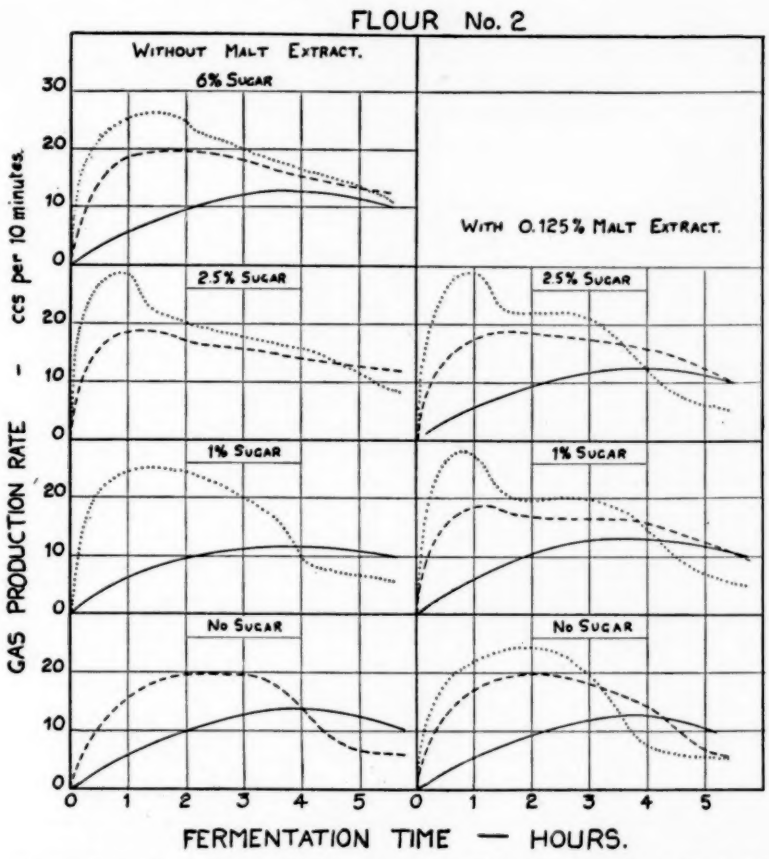

Fig. 2. Curves showing relationship between rate of CO_2 production and variation in baking formula for flour No. 2, with and without malt extract. ——— 1% yeast – – – – – 3% yeast ····· 5% yeast.

pointed out that flour No. 1, on account of its low diastatic activity, was barely able to maintain its CO_2 production above the minimum adequate rate of 10 cc. per 10 minutes until the end of the proving period, 3 hours 55 minutes, with 3% yeast and 2.5% sugar. In the latter part of the proving period, the rate was falling quite rapidly. Either lowering the sugar or raising the yeast in the formula leaves the dough with insufficient gas for proper proving. This flour represents the case where the standard formula barely fits the requirement in respect to gas production. Obviously any attempt to measure fermentation tolerance with the idea of getting an estimate of strength would lead to very erroneous conclusions. Flour No. 2, on the other hand, on account of its high diastatic activity maintained an adequate gas rate with the standard basic formula for more than 5 hours, and during the regular proving period, from the 3d to the 4th hour after mixing, the rate was maintained well above the minimum requirement.

TABLE I(*a*)

Loaf Volumes and CO_2 Production by Different Formulas Using Flour No. 1 —an Experimentally Milled High Protein Composite

2 hours fermentation (+55 minutes proofing)

Formula		Loaf volumes								CO_2 production data			
										Basic		Malt	
No.	Description	Basic	Bromate	Bromate response	Malt	Malt-bromate	Bromate response	Malt response	Response to malt with $KBrO_3$	Average rate in proof	Total CO_2 to oven time	Average rate in proof	Total CO_2 to oven time
		Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*
1a	1% yeast + no sugar	480	528	48	520	610	90	40	82	10.5	145	9.0	145
1b	" " + 1% "	490	585	95	510	650	140	30	65	9.5	134	8.0	113
1c	" " + 2.5% "	490	580	90	550	648	98	60	68	10.3	140	8.3	111
1d	" " + 6% "	515	610	95	580	635	55	65	25	9.0	113	8.2	104
2a	3% yeast + no sugar	615	685	70	710	810	100	95	125	7.0	226	16.0	234
2b	" " + 1% "	613	750	137	655	820	165	42	70	13.5	266	15.0	244
2c	" " + 2.5% "	635	715	180	668	790	122	33	75	15.5	285	13.5	244
2d	" " + 6% "	715	840	125	758	930	172	43	90	17.0	288	16.5	233
3a	5% yeast + no sugar	450	535	85	745	840	95	295	305	2.0	216	17.2	292
3b	" " + 1% "	568	755	187	760	910	150	192	155	5.0	271	17.8	299
3c	" " + 2.5% "	635	790	155	690	855	165	55	65	11.0	336	14.5	314
3d	" " + 6% "	780	905	125	850	975	125	70	60	19.7	402	20.0	368

TABLE I (*b*)

Loaf Volumes and Co_2 Production by Different Formulas Using Flour No. 1— an Experimentally Milled High Protein Composite

3 hours fermentation (+55 minutes proofing)

Formula		Loaf volumes								CO_2 production data			
										Basic		Malt	
No.	Description	Basic	Bromate	Bromate response	Malt	Malt-bromate	Bromate response	Malt response	Response to malt with $KBrO_3$	Average rate in proof	Total CO_2 to oven time	Average rate in proof	Total CO_2 to oven time
		Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*
4a	1% yeast + no sugar	565	673	108	593	710	117	28	37	9.3	211	10.5	168
4b	" " + 1% "	550	705	155	580	740	160	30	35	11.5	203	10.2	174
4c	" " + 2.5% "	565	730	165	593	725	132	28	−5	11.5	209	9.2	166
4d	" " + 6% "	595	755	160	603	735	132	8	−20	10.7	175	10.0	163
5a	3% yeast + no sugar	390	490	100	648	775	127	258	285	2.2	239	11.7	304
5b	" " + 1% "	475	645	170	693	870	177	218	225	2.7	282	14.8	333
5c	" " + 2.5% "	573	760	187	648	825	177	75	65	11.5	360	12.3	318
5d	" " + 6% "	690	905	215	760	925	165	70	20	14.5	375	15.2	324
6a	5% yeast + no sugar	370	460	90	485	580	95	115	120	2.0	228	5.0	322
6b	" " + 1% "	383	510	127	583	595	12	200	85	2.0	283	9.7	357
6c	" " + 2.5% "	445	663	218	688	895	207	243	232	—	—	14.5	401
6d	" " + 6% "	710	950	240	785	955	170	75	5	15.3	494	16.5	467

TABLE I (*c*)

LOAF VOLUMES AND CO_2 PRODUCTION BY DIFFERENT FORMULAS USING FLOUR NO. 1—AN EXPERIMENTALLY MILLED HIGH PROTEIN COMPOSITE

4 hours fermentation (+55 minutes proofing)

Formula		Loaf volumes								CO_2 production data			
										Basic		Malt	
No.	Description	Basic	Bromate	Bromate response	Malt	Malt-bromate	Bromate response	Malt response	Response to malt with $KBrO_3$	Average rate in proof	Total CO_2 to oven time	Average rate in proof	Total CO_2 to oven time
		Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*
7a	1% yeast + no sugar	560	648	88	608	765	157	48	117	3.0	230	11.0	234
7b	" " + 1% "	550	728	178	605	740	135	55	12	10.3	265	9.3	234
7c	" " + 2.5% "	555	690	135	593	790	197	38	100	10.0	269	9.2	221
7d	" " + 6% "	598	780	182	670	800	130	72	20	9.7	233	9.8	222
8a	3% yeast + no sugar	370	440	70	460	568	108	90	128	2.0	280	4.3	330
8b	" " + 1% "	395	468	83	558	730	172	73	262	2.0	290	7.7	378
8c	" " + 2.5% "	510	665	155	650	855	205	140	190	4.0	383	11.3	286
8d	" " + 6% "	685	850	165	725	870	145	40	20	11.3	453	13.2	403
9a	5% yeast + no sugar	370	435	65	448	545	97	78	110	2.0	240	3.8	345
9b	" " + 1% "	370	455	85	460	575	115	90	120	2.0	290	4.0	381
9c	" " + 2.5% "	380	470	90	548	660	112	168	190	2.0	—	9.3	457
9d	" " + 6% "	648	745	97	715	845	130	67	100	9.5	551	12.5	542

TABLE II (*a*)

LOAF VOLUMES AND CO_2 PRODUCTION BY DIFFERENT FORMULAS USING FLOUR NO. 2—AN EXPERIMENTALLY MILLED LOW PROTEIN COMPOSITE

2 hours fermentation (+55 minutes proofing)

Formula		Loaf volumes			CO_2 production data	
					Basic	
No.	Description	Basic	Bromate	Bromate response	Average rate in proof	Total CO_2 to oven time
		Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*
1a	1% yeast + no sugar	508	555	+47	11.3	142
1b	" " + 1% "	515	525	+10	10.0	142
1c	" " + 2.5% "	525	548	+23	—	—
1d	" " + 6% "	533	530	−3	10.2	131
2a	3% yeast + no sugar	585	558	−27	19.0	295
2b	" " + 1% "	590	538	−52	—	—
2c	" " + 2.5% "	585	545	−40	16.3	301
2d	" " + 6% "	603	535	−68	17.7	300
3a	5% yeast + no sugar	580	563	−17	—	—
3b	" " + 1% "	585	538	−47	20.8	392
3c	" " + 2.5% "	560	535	−25	18.7	390
3d	" " + 6% "	570	555	−15	20.3	405

TABLE II (*b*)

LOAF VOLUMES AND CO_2 PRODUCTION BY DIFFERENT FORMULAS USING FLOUR NO. 2—AN EXPERIMENTALLY MILLED LOW PROTEIN COMPOSITE

3 hours fermentation (+55 minutes proofing)

Formula		Loaf volumes			CO_2 production data: Basic	
No.	Description	Basic	Bromate	Bromate response	Average rate in proof	Total CO_2 to oven time
		Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*
4a	1% yeast + no sugar	550	555	+5	12.8	219
4b	" " + 1% "	573	575	+2	11.5	211
4c	" " + 2.5% "	573	565	−8	—	—
4d	" " + 6% "	570	575	+5	12.0	203
5a	3% yeast + no sugar	560	540	−20	16.7	393
5b	" " + 1% "	573	555	−18	—	—
5c	" " + 2.5% "	580	563	−17	15.0	391
5d	" " + 6% "	613	568	−45	16.0	396
6a	5% yeast + no sugar	488	470	−18	—	—
6b	" " + 1% "	543	515	−28	13.8	475
6c	" " + 2.5% "	538	530	−8	16.3	488
6d	" " + 6% "	563	555	−8	17.3	509

TABLE II (*c*)

LOAF VOLUMES AND CO_2 PRODUCTION BY DIFFERENT FORMULAS USING FLOUR NO. 2—AN EXPERIMENTALLY MILLED LOW PROTEIN COMPOSITE

4 hours fermentation (+55 minutes proofing)

Formula		Loaf volumes			CO_2 production data: Basic	
No.	Description	Basic	Bromate	Bromate response	Average rate in proof	Total CO_2 to oven time
		Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*
7a	1% yeast + no sugar	580	545	−35	12.2	292
7b	" " + 1% "	565	500	−65	10.8	276
7c	" " + 2.5% "	555	515	−40	—	—
7d	" " + 6% "	568	535	−33	11.0	269
8a	3% yeast + no sugar	505	495	−10	8.2	445
8b	" " + 1% "	558	500	−58	—	—
8c	" " + 2.5% "	543	495	−48	13.0	469
8d	" " + 6% "	563	510	−53	13.7	478
9a	5% yeast + no sugar	450	420	−30	—	—
9b	" " + 1% "	445	440	−5	7.3	517
9c	" " + 2.5% "	510	450	−60	13.0	566
9d	" " + 6% "	538	485	−53	14.3	597

The result of these conditions can be seen in Tables I and II, by referring to the 3% yeast + 2.5% sugar formula. For convenience these data are segregated in Table III.

TABLE III

LOAF VOLUMES BY THE BASIC FORMULA AT 2, 3, AND 4 HOURS FERMENTATION TIME

Flour No.	Loaf volume		
	2 hours	3 hours	4 hours
	Cc.	*Cc.*	*Cc.*
1	635	573	510
2	585	580	543

On the basis of these results, one might be justified in concluding that flour 2 had greater tolerance to fermentation than flour 1. Leaving aside the factor of fermentation time and considering only the loaf volume by the standard three hour fermentation, there is practically no differentiation between these two flours. The crust color in both cases was good, and while it is true that No. 1 showed the coarser texture, this fact alone would provide small indication of the great inherent difference between them. Cases similar to this are of very frequent occurrence in dealing with Canadian hard red spring wheats. The standard basic formula, satisfactory though it may be for testing commercial flours, falls down badly in the differentiation of hard wheats. This is not to be construed as a condemnation of the standard baking test as a whole, but merely of the use of one of its formulas for testing wheat.

The Relation between Formula and Fermentation Tolerance

As the data in Tables I and II are rather difficult to handle so as to bring out the essential points, some of the more pertinent data are presented as graphs in Figures 3 and 4. Figure 3 shows loaf volume by 24 formulas and 3 procedures in which the fermentation time was varied for flour No. 1, with the loaf volume range of flour No. 2 lined in for comparison. Figure 4 shows loaf volumes for flour No. 1 by the same formulas and procedures, but with 0.125% high diastatic malt in each formula. Flour No. 2 was not baked by the malt formulas because the gas production rates were not materially affected by malt, and further because the supply of flour was limited. The ranges shown in Figure 4 for flour No. 2 are the same as shown in Figure 3. While this comparison is recognized as not wholly valid, we think that it may be useful in some respects. All these curves are fermentation tolerance curves. As both bromated and non-bromated formulas were used in these studies, the bromate differential must also be considered, more particularly with respect to the time factor.

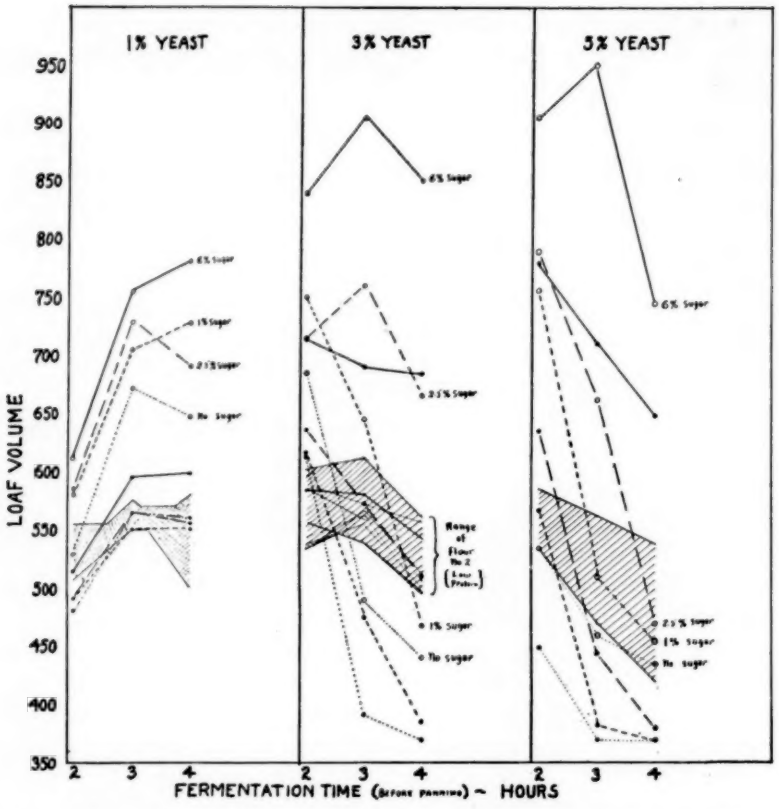

Fig. 3. Graph showing relationship between time of fermentation and loaf volume with variation in baking formula for flour No. 1 *without malt extract*. The shaded portions show the same relationships for flour No. 2.

Considering the strong flour first, it should be noted that good fermentation tolerance was shown with all the 1% yeast formulas, with only the high sugar + 3% yeast formulas, and with none of the 5% yeast formulas. With 1% yeast, sugar is not a limiting factor at four hours fermentation, except in the case of the no sugar formula. In all cases, this flour gave positive response to bromate. Furthermore, as the intensity of the fermentation increased through use of more yeast, the loaf volume range increased; the spread downward being due to exhaustion of sugar, and the upward spread being due to higher gas rates during the proof.

Flour No. 2 behaved altogether differently. It showed evidences of good fermentation tolerance only with non-bromated doughs and 1% yeast. Reference to Table II shows that with 1% yeast and 2 hours fermentation there was positive differentiation attributed to bromate, while with the 4 hour fermentation the bromate differential was negative.

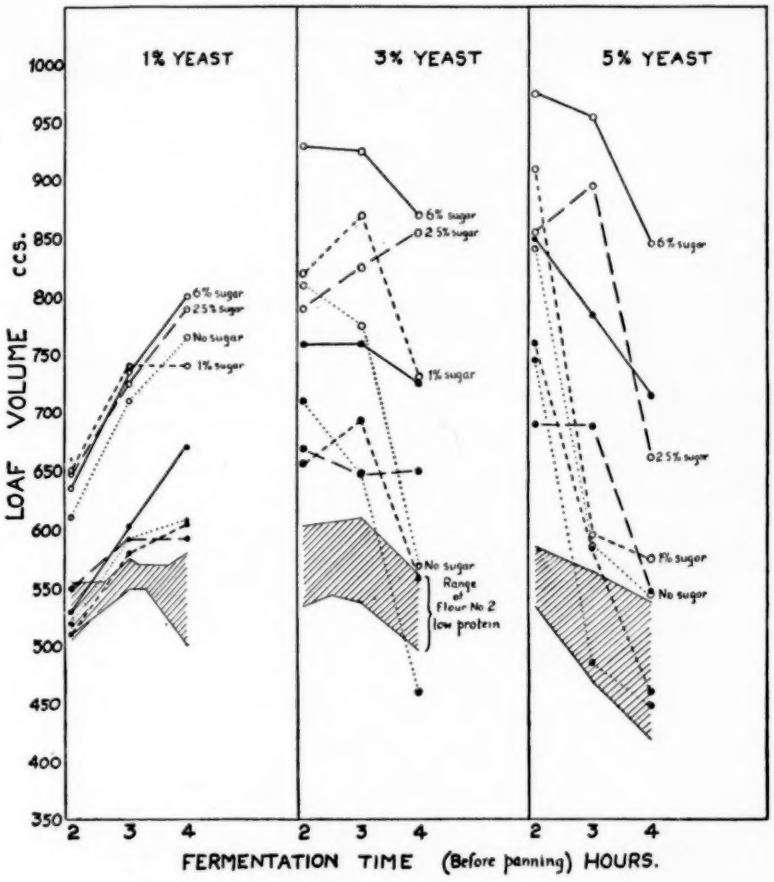

Fig. 4. Graph showing relationship between time of fermentation and loaf volume with variation in baking formula for flour No. 1 *with 0.125% malt extract.* The shaded portions show the same relationships for flour No. 2 without malt extract.

With the 3% and 5% yeast the bromate differential was negative in all cases. The gas production rate in the proof period was 10 cc. per 10 minutes or higher in all cases except the formulas 3% yeast + no sugar, 5% yeast + no sugar, and 5% yeast + 1% sugar with 4 hours fermentation, and likely, with 5% yeast + no sugar and 3 hour fermentation, and 3% yeast + 1% sugar with 4 hour fermentation. Actual data are not available for the gas production by these last two formulas, but from consideration of the loaf volumes in the series the assumption made above is likely valid. With the exception of these five formulas, the gas production rate during proving could not be considered a limiting factor in the ordinary sense, and therefore it must be assumed that the limit of

fermentation tolerance for flour No. 2 is not more than 1 hour beyond a two hour fermentation with 3% or 5% yeast, either with or without bromate, and with 1% yeast if bromate is used. It would, therefore, have to be considered weak in this respect.

Flour No. 1, on the other hand, had adequate gas production for proving in relatively few instances beside the 1% yeast formulas. The 2 hours fermentation with 5% yeast and both the no-sugar and 1% sugar gave low gassing rates; the three hours fermentation with 3% yeast and 1% sugar was inadequate, while with 5% yeast only the 6% sugar level gave sufficient gassing; with the four hour fermentation it required 6% sugar for both 3% and 5% yeast. This flour evidently required a supplement of diastase. The whole series of bakings and CO_2 production measurements was repeated using 0.125% of 200° Lintner malt extract in addition to the other ingredients. The results are given in Table I and Figure 4. This addition of malt raised the CO_2 production during the proof period to the minimum adequate rate of 10 cc. per 10 minutes for all except some of the high yeast-low sugar formulas. The standard formula, 3% yeast + 2.5% sugar with 3 hours fermentation, with 0.001% $KBrO_3$ and 0.125% malt extract gave results which were what we expected of this flour, namely, loaf volumes which were constant or slightly increasing as the fermentation time was lengthened. All the 1% yeast formulas gave similar results. All of the high yeast formulas gave decreases after 3 hours.

Now despite these decreases flour No. 1 still produced good bread as long as the volume was not much lower than 700 cc. and therefore according to the definition it was exhibiting good fermentation tolerance over the time range studied. However, in studying flours from the standpoint of intrinsic quality, any recession from an established high point indicates either failure of the flour or inadequacy of the method. Assuming flour No. 1 to be strong and flour No. 2 to be weak (and because we are studying the baking test which itself is the criterion of strength, this is the only thing we can do), we would say that the recessions from the maxima that occurred were due, with flour No. 1 to inadequacy of method, and with flour No. 2, to weakness of the flour.

Selecting a number of these formulas with which there was a slight but distinct drop in volume beyond the 3 hour fermentation, it can be seen from Table IV that many of these drops were associated with the gas rate in the proof period, which though still high enough to be considered adequate was not as high as at some shorter fermentation time. It appears evident that loaf volume is materially influenced by differences in gassing rate during proof even when it is above the minimum ade-

quate rate. We have not enough data yet to decide whether or not there is an upper limit for rate of gassing in proof beyond which no further changes in loaf volume occur. It seems probable that at a rate of 16 to 17 cc. per 10 minutes, other factors become important in limiting loaf volume. However that may be, it is certain that all other things being equal, a gassing rate of 16 will produce a larger volume than a rate of 10.

TABLE IV

SOME COMPARISONS OF CO_2 PRODUCTION RATES IN PROOF PERIOD AND LOAF VOLUMES

Flour no.	Formula	Loaf volume			CO_2 production rate in proof		
		2 hr.	3 hr.	4 hr.	2 hr.	3 hr.	4 hr.
		Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*
1	3% yeast + 6% sugar malt and BrO_3	930	925	870	16.5	15.2	15.2
1	5% yeast + 6% sugar + malt and BrO_3	975	955	845	20.0	16.5	12.5
2	3% yeast + 6% sugar	603	613	563	17.7	16.0	13.7
2	5% yeast + 6% sugar	570	563	538	20.3	17.3	14.3
1	1% yeast + 6% sugar	515	595	598	9.0	10.7	9.8
2	1% yeast + 6% sugar	533	570	568	10.2	12.0	11.0

Proper Formulas for Fermentation Tolerance Tests

In view of the foregoing discussion it may be stated emphatically that *fermentation tolerance tests must be made with formulas that ensure a constant or increasing rate of CO_2 production in proof at each increment of time used.* A decreasing rate will be almost certain to create a maximum beyond which the volume declines although the quality of bread produced may entitle it to be still classed as "good bread."

The only formulas in this series that provide these conditions are those involving use of 1% yeast. Examination of the gas rate curves for 1% yeast in Figures 1 to 4 inclusive, leads to the conclusion that the yeast is the limiting factor in CO_2 production over a long time range, irrespective of the amount of sugar added in the formula, and that the rate, after reaching a maximum in about two hours in all cases, remains relatively constant. The best choice of the formulas herein discussed seems to be 1% yeast and 6% sugar.

Differentiation of Wheats

Whenever sugar is not a limiting factor, the amount of CO_2 produced in a given time is increased as the yeast in the formula is increased. The total CO_2 produced in a fixed time gives a measure of the fermentation intensity. It should be noted in Figure 4 that the strong flour responds to increasing intensity of fermentation whereas the weak flour shows a maximum with 3% yeast, indicating that it cannot stand heavy fermentation. This is as would be expected if our general ideas regarding strength are correct. The weak flour reaches the limit of its distensibility at a lower gassing rate than the strong flour and thereafter increases in the rate have little or no effect, while the strong flour continues expanding until some higher limit of distensibility is reached. Provided care is taken to ensure proper gluten development, the response to increased gassing rates ought to provide a good measure of the inherent gluten strength of the flour. This suggests the use of a yeast differential with all other factors constant, rather than a time differential as a basis for experimental studies of fermentation tolerance.

The objection to the time differential method for testing wheats is that if one chooses a formula such that there will be no decrease in the gassing rate during the proof period for the various times selected, it must be a low gassing formula and under these conditions the latent capacity of strong flours to respond positively to fermentation of higher intensities is not apparent.

It must be recognized that in general strong experimentally-milled flours are far more sensitive to changes in fermentation conditions than are weak ones. It must also be recognized that sensitivity to baking conditions is not desirable in finished commercial flours. Therefore there is no justification whatsoever for assuming that formulas suitable for testing commercial flours, in respect to stability under different conditions can be applied with any degree of success in testing wheats for their blending value. True differentiation of wheats requires baking methods designed to bring out all the latent capacity of the strongest samples. If in applying such methods the weaker flours suffer, there will still be obtained a better idea of relative values than when formulas which only partially develop the strong ones are employed. This is brought out more clearly in Figure 5 in which are shown the effects of varying the yeast in the 6% sugar formula with 3 hour fermentation.

The weaker flour, No. 2, gave a maximum with 3% yeast, by the basic formula; with the bromate it showed only a slight negative response to increase in yeast. The stronger flour, No. 1, gave positive responses to yeast with each of the four formulas used. Taking the

difference between the 1% yeast and the 5% yeast formulas as the responses, they were for the basic formula 110 cc., for the malt formula 185 cc., for the bromate formula 195 cc., and for the malt-bromate formula 223 cc. But these responses expressed in this way are rather meaningless unless one has in mind the initial loaf volumes of the flours compared. It would seem more reasonable to consider how the variation in fermentation intensity, as brought about by increasing the yeast, affected the difference between the two flours. Using this basis, the differences between th loaf volumes of the two flours by the bromate formula were affected by yeast as follows: 1% yeast—180 cc., 3% yeast

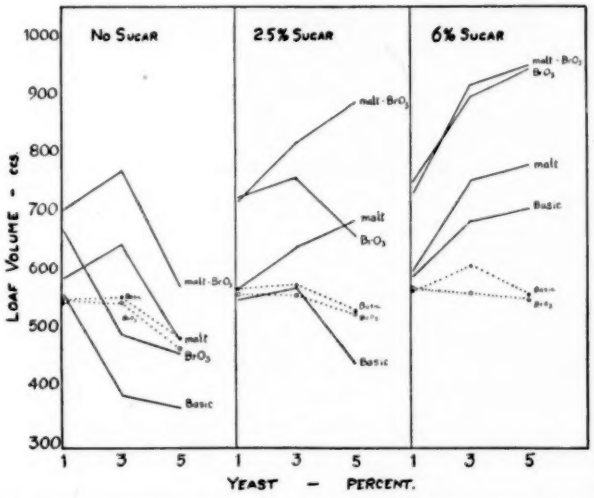

Fig. 5. Graph showing relationship between increase in yeast content in the baking formula and loaf volume, with 3 hours fermentation by the basic, bromate, malt and malt-bromate method. The full lines are for flour No. 1, the dotted lines are for flour No. 2.

—335 cc., and 5% yeast—387 cc. If in place of flour No. 2 there were substituted a standard flour of some sort, this series of changes would give a fairly clear picture of the relative tolerance of the two flours toward intense fermentation, as well as an idea of their relative placing in a loaf volume range.

Aside from the question of responses to yeast and other ingredients, the fact stands out clearly that the greatest differentiation between these samples was obtained with the 5% yeast + 6% sugar + 0.001% $KBrO_3$ formula at 3 hours fermentation time. Since the principal object of testing wheats is to differentiate them sufficiently to permit correct classification, this formula obviously is the best of those considered for this purpose.

The question naturally arises, might there not be a better formula for this purpose? We have not sufficient data on hand to justify a definite answer, but there are certain features of this formula that lend force to the conviction that the "optimum" formula for differentiation would be at least close in composition to this one. First it should be noted that with this formula there is practically no response to diastatic malt extract, which indicates that the yeast is the limiting factor in gas production, or in other words, sugar is always in excess. This can be seen more readily in Table V. These observations point to the conclu-

TABLE V

THE EFFECT OF DIASTATIC MALT EXTRACT IN THE 6% SUGAR-BROMATE FORMULA WITH 1, 3, AND 5% YEAST

Formula	1% yeast	3% yeast	5% yeast
	Loaf volume in cubic centimeters		
Bromate	755	905	950
Malt-Bromate	735	925	955
	Rate of CO_2 production in proof in cubic centimeters		
Bromate	10.7	14.5	15.3
Malt-Bromate	10.0	15.2	16.5
	Total CO_2 produced in cubic centimeters		
Bromate	175	375	494
Malt-Bromate	163	324	467

sion that further increase of sugar likely would not increase the gassing rate materially.

Secondly, it seems probable that there is an optimum gassing rate during the proof period that depends on the strength of the gluten. When the dough reaches its limit of distensibility, a greater gassing rate would cause only minor differences in final loaf volume. This assumption is supported by the behaviour of flour No. 2, which apparently reached the limit with the 3% yeast + 6% sugar formula, which was not the highest gassing formula. This can be seen by comparing formulas 3d and 5d in Table II. While further work is necessary to give definite assurance on this point, the data in hand indicate that there must be an upper limit to gassing rates, beyond which no further increase in volume can be obtained.

For the flours under consideration, therefore, the 5% yeast + 6% sugar + 0.001% $KBrO_3$—3 hour fermentation procedure comes very close to fulfilling the ideal requirements of eliminating sugar, gassing

rate, and dough development as limiting factors in the differentiation of the samples by the baking test.

Summary

The so-called "fermentation tolerance" procedure by which the behaviour of doughs fermented for varying times is observed, is of little value for estimating quality of flours experimentally milled from Canadian hard red spring wheat. Low protein flours of this class exhibit greater stability toward both change in formula and change in fermentation time than high protein flours, principally because their range of loaf volume by any procedure is small and close to the lower limit for the production of good bread. This lower limit is dependent on the maintenance of a minimum adequate gas production rate during pan-proof, and while high protein flours may develop very large volumes when the gas production rate is increased above this minimum, the low protein flours do not possess the capacity to respond to any great extent to this factor. In consequence of this, a high protein flour may drop 200 cc. in volume from 3 to 4 hours fermentation and still be 200 cc. higher than the maximum of the low protein flour, which under the same conditions may have dropped only 30–50 cc. The mere provision of sufficient diastatic supplement to eliminate diastatic activity as a limiting factor, does not solve the difficulty of using stability toward fermentation as a measure of strength. The low protein flour will nearly always exhibit great stability, and the weaker it is the greater will be its apparent stability, because the closer is its maximum value to the lower limit of acceptability, the smaller is the range through which it can fluctuate. Strong flours are usually very sensitive to changes in formula while weak flours are insensitive to them. Great stability toward changes in fermentation time and formula ingredients is usually a mark of low strength in experimentally milled flours. Fermentations carried on at high gassing rates with bromate provide the best differentiation between high and low protein flours of the type under discussion.

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THE BEHAVIOUR OF STRONG FLOURS OF WIDELY VARYING PROTEIN CONTENT WHEN SUBJECTED TO NORMAL AND SEVERE BAKING PROCEDURES [1, 2, 3]

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(Presented at the Annual Meeting, June, 1934)

Introduction

The wide use of the protein test in the United States and Canada for evaluating wheat quality, indicates that protein content is recognized as being a reliable index of strength, when the wheats under consideration are of the same class. Until recently, however, experimental test baking procedures had not been developed which would reveal a very significant relationship between these two variables, particularly in the instance of flours experimentally milled from sound high protein hard red spring wheats. In studying this phase of the question, Larmour (1930) concluded that correlation coefficients for wheat protein and quality, as measured by the bromate baking method, were in practically all cases sufficiently high to warrant concluding that the relation is significant enough to justify the commercial use of the protein test as a factor in the classification of hard red spring wheat. In subsequent tests (1931) he found a correlation of a much higher order when, by using a blend-bromate formula containing 50% of a low protein soft flour, the regression was linear over the whole range in protein content examined. Geddes and Larmour (1933), however, in an extended series of tests pertaining to the application of the basic and bromate formulas to hard red spring wheat flours, concluded that in evaluating strength in flours it is important that variations in the diastatic activity should not be a limiting factor, and extensive studies are necessary in order to determine the best means by which this may be accomplished. They further stated that it seems unlikely that a single standard baking procedure which would reveal the true strength of a series of flours can ever be devised which will show the true relative strength of a series of flours varying widely in protein content and diastatic activity.

[1] Subcommittee report, 1933–34 Committee on the Standardization of Laboratory Baking.
[2] Issued as Paper No. 6, Grain Research Laboratory, Board of Grain Commissioners for Canada.
[3] Published as paper No. 54 of the Associate Committee on Grain Research, National Research Council of Canada.

It is recognized, however, that the ability of a flour to produce a loaf of large volume and good texture under normal baking conditions is only one phase of baking strength, and it is generally understood that the stronger the flour the greater is its capacity to withstand severe baking conditions involving extended mixing or prolonged fermentation. For example, Swanson (1928) reported that the mechanical modification of dough by severe and extended mixing detects any weakness inherent in the gluten and that this method will reveal greater differences than the conventional baking procedures. The importance attached to fermentation tolerance or ability to produce good bread over a wide range in fermentation time has recently been emphasized by Fisher (1933) who points out that a strong flour which will produce good bread over a long period is obviously more fool-proof, requires less care and attention during fermentation, is less likely to go wrong in the event of accident or inadvertence, and is consequently worth more to the baker than a weaker flour.

In consideration of the importance of these widely accepted strength characteristics, namely tolerance to overfermentation and overmixing, the investigation reported at this time was undertaken for two reasons: (1) to determine the relation between protein content and capacity to withstand severe baking conditions, and (2) to ascertain the baking formulas and procedures which would reveal the maximum loaf volume differentiation in a series of flours of widely varying protein content.

In a study of this nature it appeared desirable to employ samples which would be as free as possible from any contributing factor other than protein content; also if the results are to be considered in the light of commercial application, to work with actual commercial samples rather than with pure varieties of known origin, since commercial wheats as a rule are composites comprising numerous individual wheats drawn from a large number of districts.

Experimental

In 1932, arrangements were made to extend the protein survey, which is conducted each year by the Dominion Grain Research Laboratory to include, among other things, a study of protein content in relation to baking quality. In that year more than 12,000 individual samples from approximately 2,000 shipping points in the three prairie provinces were tested in the survey and the residues from these were blended according to corresponding grade and protein content to provide ten composites, varying in protein content from 12.1% to 16.5% in increments of approximately 0.5%. By confining the individual samples in the blends to Nos. One Hard and One Northern, grades known to be

free from all forms of damage and containing varieties equal to Marquis in quality, a series was made available which was considered to be quite uniform in protein quality, especially in view of the large number of small samples comprising each composite. With such a set of samples, protein content was regarded as the only major variable.

Even though the number of composites is comparatively small, by virtue of their composition, the results obtained may be considered as representing those of the average of the individual samples. Larmour and Brockington (1931) have shown this to be a logical supposition and recommend such a practice, since it permits the use of a larger number of baking procedures, thus giving a more complete and comprehensive estimate of the character of the wheat in hand.

Reference to Table I shows the protein content of the ten composites and the corresponding flours, the diastatic activity of the flours and the approximate number of individual samples comprising each composite. The latter figures were calculated from data showing the distribution in 0.5% increments for the entire protein range covered in the 1932 survey.

TABLE I

PROTEIN CONTENT OF THE TEN COMPOSITES AND THE CORRESPONDING EXPERIMENTALLY MILLED STRAIGHT GRADE FLOURS; DIASTATIC ACTIVITY OF THE FLOURS, AND THE APPROXIMATE NUMBER OF INDIVIDUAL SAMPLES COMPRISING EACH COMPOSITE

Protein content (13.5% Moisture basis)		Diastatic activity [1]	Approximate number of individual samples
Wheat P.ct.	Flour P.ct.	(mg. maltose per 10 gms. flour)	
16.5	15.7	104	170
16.1	15.4	110	360
15.6	14.7	110	720
14.7	14.2	110	1050
14.4	13.6	116	1280
13.8	13.1	110	1230
13.4	12.8	114	980
13.0	12.3	94	700
12.3	11.8	88	520
12.1	11.4	100	530

[1] The diastases and the maltose figures were determined according to the method outlined by Blish, M. J. 1933. J. Assoc. Official Agr. Chem. Vol. XVI, No. 4: 497.

Attention is directed to the very small range in diastatic activity for all ten flours, the maximum difference being only 28 mg., indicating that in this particular series, variations due to diastatic activity do not materially enter into the baking results.

The wheats were experimentally milled to straight grade flours of approximately 70% extraction and baked by 24 methods varying in

either formula or procedure selected to determine the behaviour of the flours over a wide range of baking conditions. The baking methods employed are outlined below:

(*A*) Procedures Involving a Fixed Fermentation Time of Three Hours with Various Formulas

1. *Basic*—A formula approximating that of the A. A. C. C. basic procedure excepting that the absorption was varied to suit the requirements of the flour, the dough machine mixed (Hobart, 3 mins. medium speed) and baked in a low-sided tin.
2. *Malt-Phosphate*—Basic formula plus 0.3 gm. diastatic malt (approx. 200° Lintner) and 0.1 gm. $NH_4H_2PO_4$.
3. *Bromate*—Basic formula plus 0.001 gm. $KBrO_3$.
4. *Malt-Phosphate-Bromate*—Basic formula plus 0.3 gm. diastatic malt, 0.1 gm. $NH_4H_2PO_4$ and 0.001 gm. $KBrO_3$.

(*B*) Procedures Designed to Determine Tolerance to Bromate

5. *Malt-Phosphate-Bromate Formulas*—Same as (2) plus the following amounts of potassium bromate: 0.001, 0.002, 0.003 and 0.004 gm.
6. *Bromate Formulas*—Basic formula plus 0.001, 0.002, 0.003 and 0.004 gm. $KBrO_3$.

(*C*) Procedures Designed to Determine Tolerance to Fermentation

7. *Malt-Phosphate-Bromate Formula* (4) employing fermentation times of 2, 3, 4 and 5 hours.

(*D*) Procedures Designed to Determine Tolerance to Mixing

8. *Malt-Phosphate Formula* (2) with doughs mixed 1, 3, 6 and 9 minutes in the Hobart-Swanson mixer.
9. *Malt-Phosphate-Bromate Formula* (4) with doughs mixed for 1, 3, 6 and 9 minutes as above.

(*E*) Procedures Involving Dilution with Low Protein Flour (English 8.9%)

10. *Malt-Phosphate-Bromate Formula* (4) with flours under study diluted with 15% and 30% English flour respectively.

Experimental Baking Results

While the absorptions were recorded and all loaves judged for symmetry, crust colour, crumb texture, and colour, the discussion of the results will be, for the sake of simplicity, restricted in the main to the loaf volume data. It should be mentioned, however, that the absorption increased with the protein content and that, for any given baking procedure, the internal loaf characteristics were closely related to loaf volume. A consideration of the relative loaf volumes yielded by the different flours under varying baking conditions therefore satisfactorily reflects the comparative baking quality of this class of experimental material. The data are considered in the order in which the baking

formulas are outlined. In addition to presenting the absolute mean loaf volumes, these have been expressed on two percentage bases—one in which the values for the different flours are expressed as a percentage of that given by the highest protein flour baked by the same procedure, and the other in which the several volumes for each flour obtained by different baking methods are expressed as a percentage of the values given by one particular procedure selected as a standard. By this means the absolute and relative responses of the different flours to varying baking conditions may be readily observed.

(A) Results by Formulas Employing a Three-hour Fermentation Time

The results by the basic, malt-phosphate, bromate and malt-phosphate-bromate formulas, employing a fixed fermentation time of three hours, are recorded in Table II, and represented graphically in Figure 1.

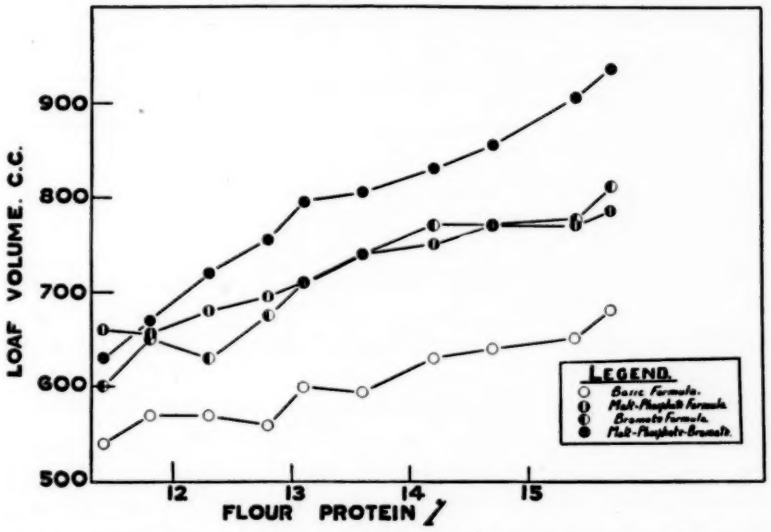

Fig. 1. Showing comparative mean loaf volumes given by strong flours of varying protein content when baked by the basic, malt-phosphate, bromate and malt-phosphate-bromate formulas, employing a 3-hour fermentation time.

It will be observed that there is a close relationship between loaf volume and protein content with all four formulas and that the flours, with few exceptions, would be similarly classified in regard to strength in each case. With all ten flours, the basic formula gave the lowest loaf volumes and the malt-phosphate-bromate formula the highest, while the malt-phosphate and the bromate gave approximately similar values intermediate to these. It is interesting to note that the basic loaf volume for the 15.7% protein flour is approximately equal to that of the 11.8% protein flour by the malt-phosphate-bromate formula. In regard to

TABLE II

MEAN LOAF VOLUME DATA FOR THE BASIC, MALT-PHOSPHATE, BROMATE AND MALT-PHOSPHATE-BROMATE FORMULAS EMPLOYING A FIXED FERMENTATION TIME OF 3 HOURS

Flour protein	Basic			Malt-Phosphate			Bromate			Malt-Phosphate-Bromate		
(13.5% M.B.) *P.ct.*	Loaf volume			Loaf volume			Loaf volume			Loaf volume		
	Cc.	%(*1*)	%(*2*)	*Cc.*	%(*1*)	%(*2*)	*Cc.*	%(*1*)	%(*2*)	*Cc.*	%(*1*)	%(*2*)
15.7	680	100	100	785	100	115	810	100	119	935	100	138
15.4	650	96	100	770	98	118	775	96	119	910	97	140
14.7	640	94	100	770	98	120	770	95	120	855	91	134
14.2	630	93	100	750	96	119	770	95	122	830	89	132
13.6	595	88	100	740	94	124	740	91	124	805	86	135
13.1	600	88	100	710	90	118	710	88	118	795	85	132
12.8	560	82	100	695	88	124	675	83	120	760	81	136
12.3	570	84	100	680	87	119	630	78	110	720	77	126
11.8	570	84	100	655	83	115	650	80	114	670	72	118
11.4	540	79	100	660	84	122	600	74	111	630	67	117
Range	140	21	—	125	16	—	210	26	—	305	33	—

%(*1*) = Loaf volume as a percentage of the highest protein flour baked by the corresponding procedure.
%(*2*) = Loaf volume as a percentage of the corresponding flour baked by the basic procedure.

both absolute and relative differentiation in loaf volume, the formulas would be arranged in the order of malt-phosphate-bromate, bromate, basic, and malt-phosphate, the percentage differentiation amounting to 33, 26, 21, and 16 respectively.

While all the flours gave positive responses to the addition of the supplementary ingredients, there is no consistent relation between response and protein content, excepting when both malt-phosphate and bromate were employed. In this instance, the absolute and relative responses of the highest protein flour are 255 cc. and 38% as compared with 90 cc. and 17% respectively for the lowest protein flour. Of the formulas under consideration, the malt-phosphate-bromate formula, which provides for adequate gas production and also development of the gluten, is preferable from the standpoint of providing large absolute volumes and maximum differentiation between samples.

(*B*) *Tolerance to Bromate*

The loaf volumes obtained by increasing increments of bromate alone and when superimposed upon malt-phosphate, are shown in Table III and represented graphically in Figures 2 and 3.

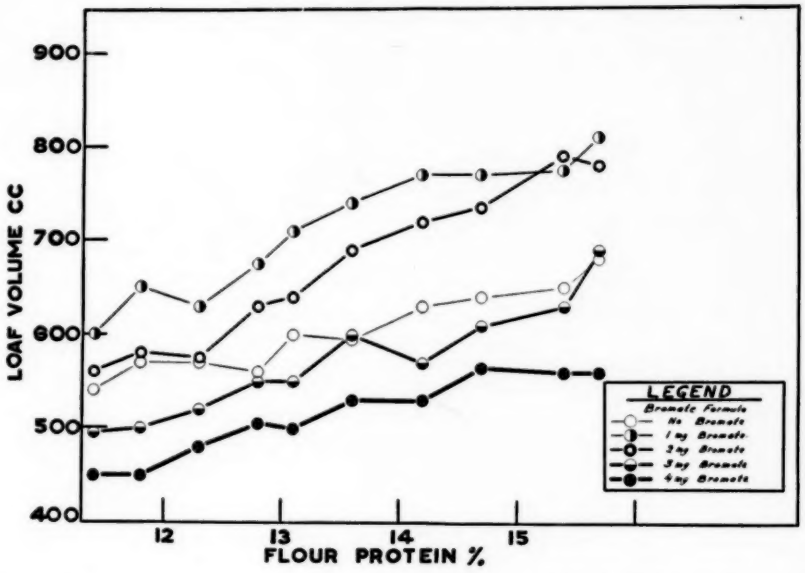

Fig. 2. Showing comparative mean loaf volumes given by strong flours of varying protein content when baked with increasing increments of bromate, employing a 3-hour fermentation period.

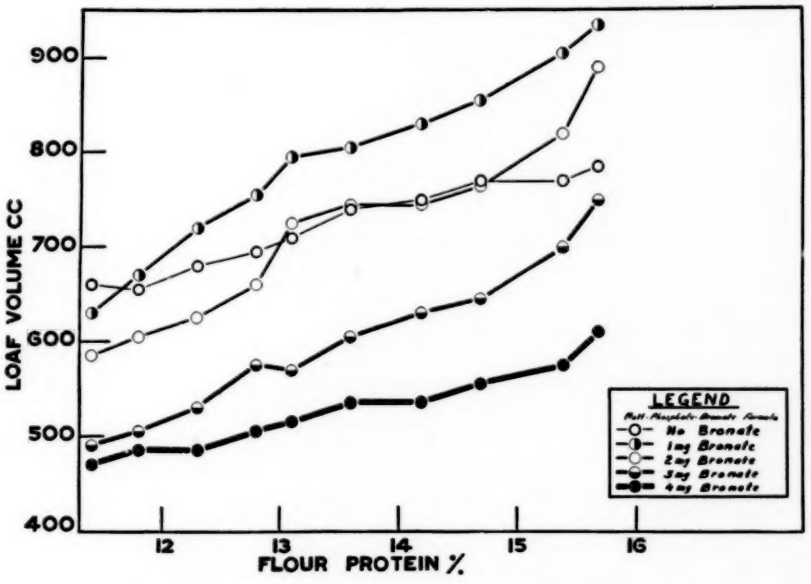

Fig. 3. Showing comparative mean loaf volumes given by strong flours of varying protein content when baked with increasing increments of bromate plus malt-phosphate, employing a 3-hour fermentation period.

TABLE III

MEAN LOAF VOLUME DATA OBTAINED BY INCREASING INCREMENTS OF BROMATE ALONE AND WHEN SUPERIMPOSED UPON MALT-PHOSPHATE WITH A 3-HOUR FERMENTATION PERIOD

Flour protein (13.5% M.B.) P.ct.	No bromate			1 mg. bromate			2 mg. bromate			3 mg. bromate			4 mg. bromate		
	Loaf volume			Loaf volume			Loaf volume			Loaf volume			Loaf volume		
	Cc.	%(1)	%(2)	Cc.	%(1)	%(2)	Cc.	%(1)	%(2)	Cc.	%(1)	%(2)	Cc.	%(1)	%(2)
						(a) *Bromate Formula*									
15.7	680	100	100	810	100	119	780	100	115	690	100	101	560	100	82
15.4	650	96	100	775	96	119	790	101	122	630	91	97	560	100	86
14.7	640	94	100	770	95	120	735	94	115	610	88	95	565	101	88
14.2	630	93	100	770	95	122	720	92	114	570	83	90	530	95	84
13.6	595	87	100	740	91	124	690	88	116	600	87	101	530	95	89
13.1	600	88	100	710	88	118	640	82	107	550	80	92	500	89	83
12.8	560	82	100	675	83	120	630	81	112	550	80	98	505	90	90
12.3	570	84	100	630	78	110	575	74	101	520	75	91	480	86	84
11.8	570	84	100	650	80	114	580	74	102	500	72	88	450	80	79
11.4	540	79	100	600	74	111	560	72	104	495	72	92	450	80	83
Range	140	21	—	210	26	—	220	28	—	195	28	—	110	20	—
						(b) *Malt-Phosphate-Bromate Formula*									
15.7	785	100	100	935	100	119	890	100	113	750	100	96	610	100	78
15.4	770	98	100	910	97	118	820	92	106	700	93	91	575	94	75
14.7	770	98	100	855	91	111	765	86	99	645	86	84	560	92	73
14.2	750	96	100	830	89	111	745	84	99	630	84	84	535	88	71
13.6	740	94	100	805	86	109	745	84	101	610	81	82	540	88	73
13.1	710	90	100	795	85	112	725	81	102	570	76	80	515	84	72
12.8	695	88	100	760	81	109	660	74	95	575	77	83	505	83	73
12.3	680	87	100	720	77	106	625	70	92	530	71	78	485	80	71
11.8	655	83	100	670	72	102	605	68	92	505	67	77	485	80	74
11.4	660	84	100	630	67	95	580	65	88	490	65	74	470	77	71
Range	125	16	—	305	33	—	310	35	—	260	35	—	140	23	—

%(1) = Loaf volume as a percentage of the highest protein flour baked by the corresponding procedure.
%(2) = Loaf volume as a percentage of the corresponding flour containing no bromate. (Response.)

The flours are again classified in order of strength according to decreasing protein content by all the formulas, the highest absolute values being obtained with the malt-phosphte formula containing 0.001 gm. $KBrO_3$, followed by that containing 0.002 gm. $KBrO_3$. For corresponding bromate dosages the formula containing malt-phosphate gave the largest volumes for all flours. In regard to differentiation in loaf volume, the 2 and 3-milligram dosages both with and without malt-

phosphate gave the greatest maximum percentage differentiation between samples. With the 4-milligram dosage, the spread in loaf volume of the several flours narrows, due to the greater reduction in the volume of the higher protein flours. For example, with the formula containing malt-phosphate the volumes for the 15.7% protein flour are 890, 750, and 610 cc. for 2, 3, and 4 milligrams $KBrO_3$ respectively, while the corresponding values for the 11.4% protein flour are 580, 490, and 470 cc., the total reduction for the high protein flour thus being 280 cc., and that for the low protein flour, only 110 cc. In general, the absolute response to bromate in the presence of malt-phosphate with all bromate dosages is fairly closely related to the protein content, the higher protein flours giving the greater response and showing a more rapid reduction when an excess is added.

(*C*) *Results for Varying Fermentation Times*

The loaf volume data for the malt-phosphate-bromate formula using fermentation times of 2, 3, 4, and 5 hours, are shown in Table IV and Figure 4.

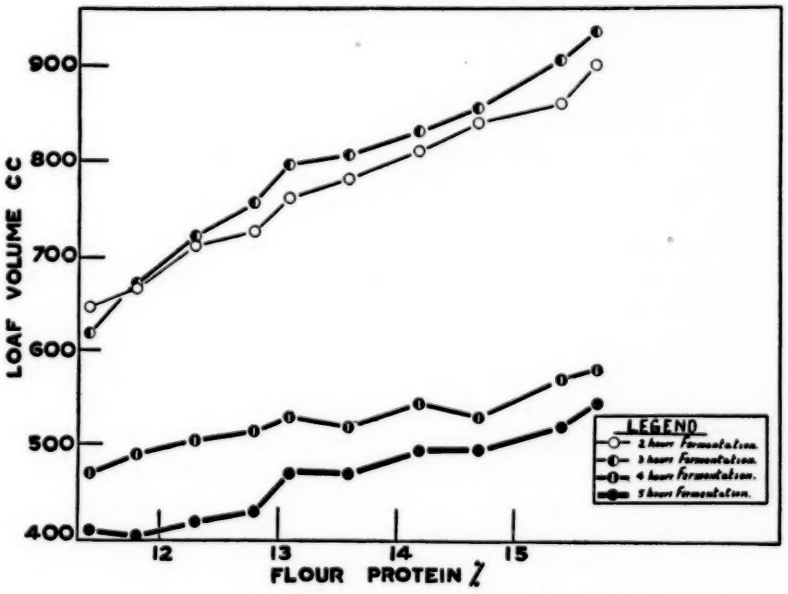

Fig. 4. Showing comparative loaf volumes given by strong flours of varying protein content when baked by the malt-phosphate-bromate formula using fermentation times of 2, 3, 4, and 5 hours.

Again, all procedures classified the strength of the flours in order of decreasing protein content, the largest volumes and greatest absolute and relative differentiation being given by the three-hour fermentation period.

TABLE IV

MEAN LOAF VOLUME DATA FOR THE MALT-PHOSPHATE-BROMATE FORMULA USING FERMENTATION TIMES OF 2, 3, 4 AND 5 HOURS

Flour protein	2 hours			3 hours			4 hours			5 hours		
(13.5% M.B.) *P.ct.*	Loaf volume			Loaf volume			Loaf volume			Loaf volume		
	Cc.	%(1)	%(2)	*Cc.*	%(1)	%(2)	*Cc.*	%(1)	%(2)	*Cc.*	%(1)	%(2)
15.7	900	100	100	935	100	104	580	100	64	545	100	60
15.4	860	96	100	910	97	106	570	98	66	520	96	60
14.7	840	93	100	855	91	102	530	91	63	495	91	59
14.2	810	90	100	830	89	102	545	94	67	495	91	61
13.6	780	87	100	805	86	103	520	90	67	470	86	60
13.1	760	84	100	795	85	105	530	91	70	470	86	62
12.8	725	80	100	760	81	105	515	89	71	430	79	59
12.3	710	79	100	720	77	101	505	87	71	420	77	59
11.8	665	74	100	670	72	101	490	84	74	405	74	61
11.4	645	72	100	630	67	98	470	81	73	410	75	64
Range	255	28	—	305	33	—	110	19	—	135	25	—

%(1) = Loaf volume as a percentage of the highest protein flour baked by the corresponding procedure.
%(2) = Loaf volume as a percentage of the corresponding flour at 2 hours' fermentation time.

The 15.7% protein flour yielded a maximum loaf volume of 935 cc. at 3 hours' fermentation and a minimum of 545 cc. at 5 hours', while the 11.4% protein flour gave a maximum volume of 645 cc. at 2 hours' fermentation and a minimum of 410 cc. at 5 hours'. Thus the spread between the maximum and minimum volumes of the high protein flour was 390 cc. as compared with 235 cc. for the low protein sample.

This greater sensitivity of the highest protein flour to alterations in fermentation time is also shown by the other high protein samples in the series, and it would appear that the higher the protein the greater the fluctuation in loaf volume. The loaf volume responses to increasing fermentation time, expressed as a percentage of the value for the 2-hour fermentation, indicate that with extended fermentation the low protein flours are no more severely injured than those of higher protein content. In other words, the so-called fermentation tolerance appears to be, in general, inversely related to the protein content of the flours under study. This is shown particularly by the 4-hour fermentation period, the volume of the 11.4% protein flour being 73% of the value at 2 hours, as compared with 64% for the 15.7% protein flour. Judged only on the basis of resistance to fermentation, the low protein flours would be regarded as having greater tolerance and, from this standpoint, superior strength. Yet by every baking test employed, the strength as measured by loaf volume, decreased with the protein content. Thus a

consideration alone of the susceptibility of the flours to change in volume as a result of varying fermentation time, leads to entirely erroneous conclusions with regard to relative strength. However, it should be emphasized that even though the volumes fell off more sharply, in the instance of the high protein flours, their final volumes were still much greater than those in the lower protein ranges. Under optimum fermentation conditions, the loaves from the lowest protein flours are close to the lower limit for satisfactory bread and hence a smaller decrease is required to bring them into the unsatisfactory class. If an arbitrary lower limit in loaf volume of 525 cc. is selected, all the flours give satisfactory loaves with 2 and 3-hour fermentation times, while for the 4-hour fermentation only those in the higher protein levels are included, and for 5 hours only the 15.7% protein flour.

It should be recalled that these flours were baked with the addition of 0.3% of high diastatic malt, and were approximately equal in diastatic activity; so that the variations in response to fermentation cannot be attributed to differences in gassing power. This belief was confirmed by the uniformity in crust colour of the samples at corresponding fermentation times, being slightly pale at 4 hours and decidedly pale at 5 hours. From the results obtained it must be concluded that sensitiveness to change is a characteristic of strong experimentally milled flours and that the usual interpretation of fermentation tolerance is inapplicable to such material.. With such material the absolute loaf volume levels, rather than the relative response, must be regarded as the major criterion of strength.

(*D*) *Tolerance to Mixing*

The loaf volumes obtained by the malt-phosphate and malt-phosphate-bromate formulas for mixing times of 1, 3, 6, and 9 minutes with the Hobart-Swanson mixer, are shown in Table V and Figures 5 and 6, and require little comment, since the results indicate the same general relationships between the flours as was found with extended fermentation.

With the malt-phosphate formula the maximum differentiation is given by the 3-minute mixing, although the values are very nearly equal to those for the 1-minute period. It is interesting to note that, when bromate is also added, the greatest differentiation is obtained with the 1-minute mixing time, and that for the 6 and 9-minute periods, the absolute loaf volumes of all flours are noticeably lower than for the corresponding mixing times without bromate. The loaf volumes for the malt-phosphate-bromate formula with the 6-minute period are approximately equal to those obtained by the 9-minute mixing time without bromate. There is thus definite evidence that bromate and overmixing

TABLE V

MEAN LOAF VOLUME DATA OBTAINED BY THE MALT-PHOSPHATE AND MALT-PHOSPHATE-BROMATE FORMULAS EMPLOYING THE HOBART-SWANSON MIXER WITH MIXING TIMES OF 1, 3, 6 AND 9 MINUTES

Flour protein (13.5% M.B.) P.ct.	1 min.			3 min.			6 min.			9 min.		
	Loaf volume			Loaf volume			Loaf volume			Loaf volume		
	Cc.	%(1)	%(2)	Cc.	%(1)	%(2)	Cc.	%(1)	%(2)	Cc.	%(1)	%(2)
(a) Malt-Phosphate Formula												
15.7	785	100	100	790	100	101	615	100	78	550	100	70
15.4	770	98	100	780	99	101	590	96	77	540	98	70
14.7	770	98	100	760	96	99	550	89	71	500	91	65
14.2	750	96	100	750	95	100	560	91	75	510	93	68
13.6	740	94	100	720	91	97	560	91	76	515	94	70
13.1	710	90	100	715	90	101	565	92	80	510	93	72
12.8	695	88	100	710	90	102	560	91	80	505	92	73
12.3	680	87	100	710	90	104	560	91	82	500	91	74
11.8	655	83	100	650	82	99	545	89	83	480	87	73
11.4	660	84	100	650	82	98	540	88	82	480	87	73
Range	125	16	—	140	18	—	75	12	—	70	13	—
(b) Malt-Phosphate-Bromate Formula												
15.7	930	100	100	825	100	89	565	100	61	500	100	54
15.4	890	96	100	750	91	84	570	101	64	480	96	54
14.7	830	89	100	705	85	85	530	94	64	470	94	57
14.2	825	89	100	700	85	85	540	96	65	470	94	57
13.6	810	87	100	720	87	89	550	97	68	475	95	59
13.1	805	86	100	700	85	87	525	93	65	440	88	55
12.8	740	80	100	680	82	92	525	93	71	440	88	59
12.3	740	80	100	660	80	89	500	88	68	445	89	60
11.8	690	74	100	620	75	90	485	86	70	435	87	63
11.4	660	71	100	625	76	95	470	83	71	420	84	64
Range	270	29	—	200	24	—	95	17	—	80	16	—

%(1) = Loaf volume as a percentage of the highest protein flour baked by the corresponding procedure.
%(2) = Loaf volume as a percentage of the corresponding flour mixed for one minute.

have the same effect on gluten development as reflected by loaf volume.

The maximum differentiation was most marked at the shortest mixing time and became less as the mixing time increased. For all extended mixing times the absolute and relative reduction in loaf volume, due to mechanical development of the gluten was, in general, less for the flours of lowest protein; in fact, the ability to resist overmixing appears to be inversely related to the protein content. In other words, the lower the protein content the greater appears to be the flour's resistance tc

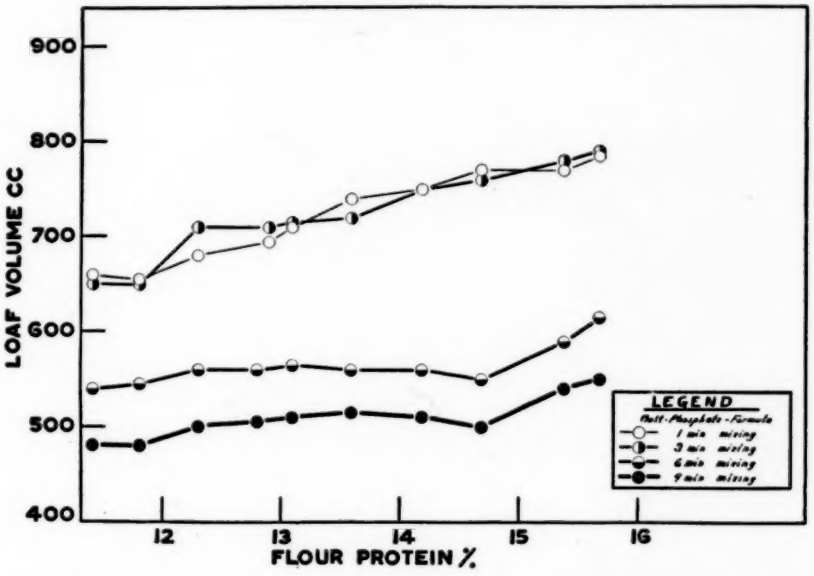

Fig. 5. Showing comparative mean loaf volumes given by strong experimentally milled flours of varying protein content when baked by the malt-phosphate formula, employing the Hobart-Swanson mixer with mixing times of 1, 3, 6, and 9 minutes.

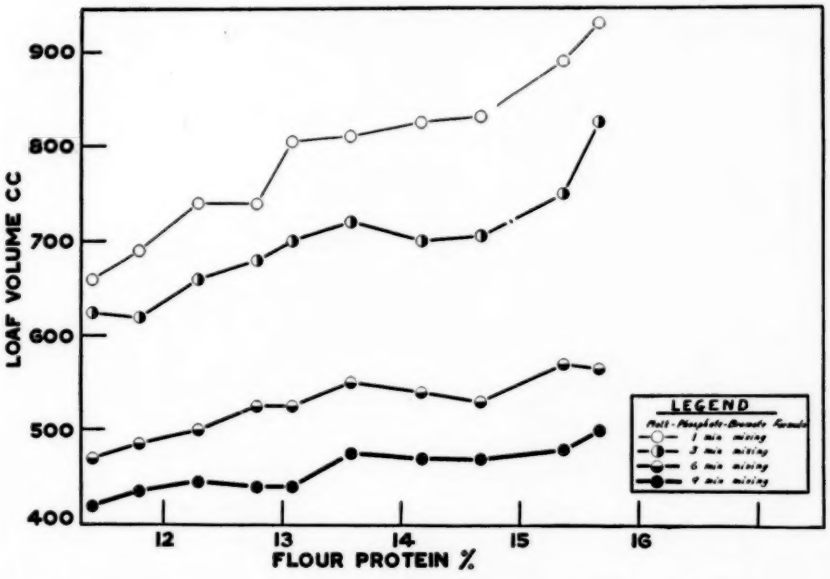

Fig. 6. Showing comparative mean loaf volumes given by strong experimentally milled flours of varying protein content when baked by the malt-phosphate-bromate formula, employing the Hobart-Swanson mixer with mixing times of 1, 3, 6, and 9 minutes.

gluten development induced by overmixing, and such a procedure would seem to be of little value in classifying strong flours such as were used in this investigation. When extended mixing is employed, the relative injury is greater for the higher protein flours than for those of lower protein content, and hence with increased severity of mixing, the loaf volumes of all flours in the series tend to approach the same level, thus reducing the differentiation between flours.

(*E*) *Tolerance to Dilution with Low Protein Flour*

The loaf volumes for the flours baked by the malt-phosphate-bromate formula alone and when diluted with 15% and 30% English low protein flour, are given in Table VI, and represented graphically in Figure 7.

TABLE VI

MEAN LOAF VOLUME DATA BY THE MALT-PHOSPHATE-BROMATE FORMULA FOR THE ORIGINAL FLOURS AND THE SAME DILUTED WITH 15 AND 30 PER CENT ENGLISH LOW PROTEIN FLOUR

Flour protein (13.5% M.B.) *P.ct.*	100% Canadian			Plus 15% English			Plus 30% English		
	Loaf volume			Loaf volume			Loaf volume		
	Cc.	%(*1*)	%(*2*)	*Cc.*	%(*1*)	%(*2*)	*Cc.*	%(*1*)	%(*2*)
15.7	935	100	100	920	100	98	820	100	88
15.4	910	97	100	870	94	96	790	96	87
14.7	855	91	100	820	89	96	740	90	86
14.2	830	89	100	815	88	98	710	86	86
13.6	805	86	100	770	84	96	705	86	88
13.1	795	85	100	730	79	92	690	84	87
12.8	760	81	100	730	79	96	670	82	88
12.3	720	77	100	695	76	96	650	79	90
11.8	670	72	100	665	72	99	610	74	91
11.4	630	67	100	620	67	98	600	73	95
Range	305	33	—	300	33	—	220	27	—

%(*1*) = Loaf volume as a percentage of the highest protein flour baked by the corresponding procedure.
%(*2*) = Loaf volume as a percentage of the corresponding flour baked by itself (100%).

With each diluted and undiluted flour, the loaf volumes are in linear relation to the protein content, the blend formulas showing no superiority in this respect. With the exception of the 13.1% protein flour, all of the flours carried 15% soft flour without materially reducing the loaf volume, the values ranging between 96% and 99% of those given by the unblended flours. When the soft flour in the blend was increased to 30%, the loaf volumes, as would be expected, were reduced by a greater amount but the lower protein flours suffered less absolute and

relative reduction than those in the higher protein ranges, resulting in a narrowing in the spread in loaf volume. For example, the 15.7% flour was reduced in volume by 115 cc. or 12%, while the 11.4% flour was reduced by only 30 cc. or 5% when diluted with 30% of the English flour. From this it might be concluded that the capacity of a flour to maintain its volume when blended with relatively high percentages of a soft low protein flour is inversely related to the protein

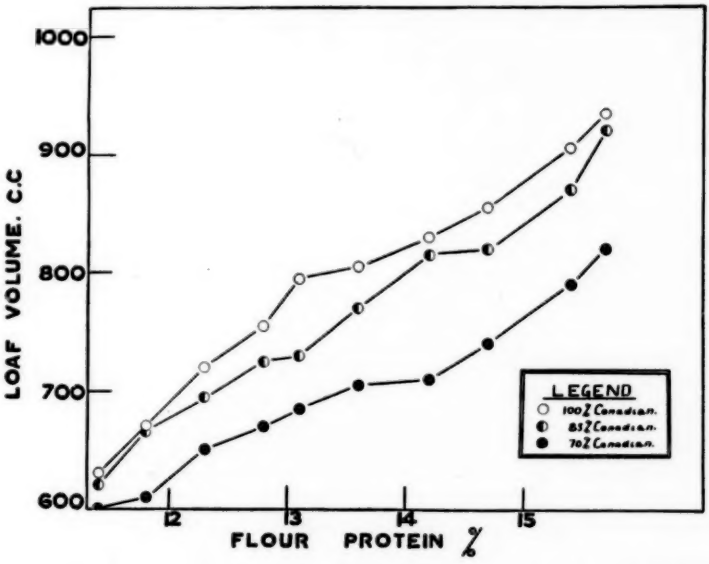

Fig. 7. Showing comparative mean loaf volumes given by strong experimentally milled flours baked alone and in blends containing 15% and 30% English low protein flour when baked by the malt-phosphate-bromate formula. (The protein content shown in the abscissa, refers to the strong flours under consideration.)

content, but as already pointed out in the instance of tolerance to fermentation, the relative loaf volumes for corresponding baking methods must be considered the index of strength, rather than the absolute or relative reduction in loaf volume by imposing more severe baking conditions.

Discussion

The application of accepted baking test procedures for evaluating flour strength to this series of strong experimentally milled flours of widely varying protein content but presumably of very similar quality, has yielded surprising results which, if interpreted on the basis of response, would lead to anomalous and erroneous conclusions. With the 24 variations in formula and procedure employed in this study, the loaf volumes, in general, bore a linear relation to flour protein over the

entire range, namely, 11.4% to 15.7%, which indicates that when protein content is the only major variable, it is a reliable index of flour strength as reflected by loaf volume. If, however, strength is judged on the ability to withstand severe baking conditions, such as the use of progressively increasing dosages of potassium bromate, extended fermentation and prolonged mixing times, and progressive dilution with weak flour, the results obtained lead to the absurd conclusion that the weaker flours in the series are the stronger, due to their relative insensitivity to the changes in baking conditions imposed.

The loaf volumes of the low protein or weaker flours in the series are, under optimum baking conditions, very close to the border line of what may be considered as a satisfactory loaf, and, while less sensitive to variations in baking procedure, the imposing of severe conditions brings them into the region of unsatisfactory bread. In contrast to this, the high protein flours yield loaves of exceptionally large volume under optimum conditions and while vacillating more widely under the same conditions, the ultimate loaf volume is at a much higher level than that of a weaker flour under corresponding conditions. In evaluating strong flours, therefore, in which protein content is the only major variable, the interpretation which should apparently be placed on responses to severe baking conditions, is the reverse of that ordinarily applied, that is, the greater the range in loaf volume the stronger the flour. In the light of these results, such tests appear to be of little value if absolute loaf volumes under corresponding baking conditions be accepted as the proper measure of relative strength, since they give less differentiation between flours than one employing a more normal procedure. Of the 24 variations in baking formula and procedure, that containing 0.3 gm. diastatic malt (approx. 200° Lintner), 0.1 gm. $NH_4H_2PO_4$ and 0.001 gm. $KBrO_3$ with a fermentation time of 3 hours, gave the highest loaf volumes for all flours, with a very high degree of differentiation.

It seems essential in determining the relative strength of hard red spring wheats, involving tests on only slightly aged experimentally milled flours, to employ an oxidizing agent in order to assist in the proper development of the gluten and, in addition, to ensure the production of sufficient gas during the proving period to give the maximum expansion of which the dough is capable.

Summary

The present investigation was undertaken to ascertain the relation between protein content and the capacity of experimentally milled hard red spring wheat flours to withstand severe baking conditions, and also the baking formulas and procedures which would reveal the maximum

loaf volume differentiation in a series of flours of widely varying protein content.

Ten composite wheats ranging in protein content from 12.1% to 16.5%, in increments of approximately 0.5%, were prepared by blending the surpluses of the samples obtained for the 1932 protein survey of Western Canadian hard red spring wheat. These wheats represented many thousands of individual samples grading Nos. One Hard and One Northern drawn from every grain growing district in the prairie provinces; and protein content is believed to be the only major variable.

The wheats were experimentally milled to straight grade flours of approximately 70% extraction and baked by 24 different formulas and procedures involving the use of the basic and several supplementary procedures. These included a malt-phosphate, bromate and malt-phosphate-bromate formula, varying mixing and fermentation times and dilution of the original flours with 15% and 30% of an English low protein flour. Loaf volume was taken as the criterion of strength since the variations in other important loaf characteristics—notably the texture—closely paralleled this variable.

With all 24 procedures, the volume, in general, increased with the protein content, the relation being approximately linear over the entire range, namely, 11.4% to 15.7%.

The absolute loaf volumes and maximum differentiation between samples was greatest with the formula containing 0.3 gm. high diastatic malt, 0.1 gm. $NH_4H_2PO_4$ and 0.001 gm. $KBrO_3$, and employing a 3-hour fermentation time.

By imposing severe baking conditions involving increasing increments of bromate, prolonged fermentation, extended mixing and progressive dilution with a low protein soft flour, the loaf volumes of the higher protein flours were reduced to a greater extent than those of lower protein content, thus decreasing the differentiation between the flours.

As reflected by loaf volume, there is definite evidence at hand to show that the addition of bromate and overmixing the dough have the same general effect on gluten development.

If insensitivity or tolerance to severe baking conditions is taken as a measure of flour strength, the flours of lowest protein content must be regarded as possessing the greatest strength. This conclusion is fallacious, since the low protein flours under optimum conditions yield loaves close to the border line of what may be considered as a satisfactory loaf and while less sensitive to variations in baking procedure, adverse conditions bring them into the region of unsatisfactory bread. On the other hand, higher protein flours which yield loaves of large volume, under optimum conditions, can suffer a greater reduction and

yield ultimate loaves of much higher volume than that of a lower protein flour baked under corresponding conditions.

The results obtained indicate that the absolute loaf volumes under corresponding baking conditions must be considered as the criterion of strength in studies of this nature.

Since the usual interpretation of tolerance is inapplicable, the use of severe baking conditions is of little value as they decrease the differentiation between flours.

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INVESTIGATION OF A MERCURY VAPOR LAMP FOR ACCELERATING THE BLEACHING ACTION OF DIBENZOYL PEROXIDE. II. QUARTZ ELECTRODE

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(Presented at the Annual Meeting, June, 1934)

Introduction

A laboratory device for accelerating the bleaching rate of dibenzoyl peroxide with radiant energy from a mercury vapor arc in Uviol glass has been described by Ferrari, Croze, and Bailey (1932). The purpose of the device was to make available the results of bleaching with the Novadel reagent soon after the bleaching treatment rather than waiting the 24 hours necessary for the complete reaction to take place. The data in this paper deal with irradiation treatments with a quartz mercury vapor arc, and differ in this respect from the experiments described in the earlier paper referred to where a Uviol glass electrode vessel was used. In other respects the equipment and the procedure were the same, and their description will not be repeated here. The quartz electrode vessel was constructed of clear, fused quartz and had substantially the same dimensions as the Uviol glass electrode. It was made available to us through the courtesy of the Wallace and Tiernan Company, Inc.

Experimental Procedure

The extent of bleaching was measured by extracting the flour with a mixture consisting of 93 parts of cleaner's naptha and 7 parts of absolute alcohol, and determining the carotinoid pigment concentration spectrophotometrically by the procedure described in detail by Ferrari, (1933). For convenience the total concentration of carotinoid pigments is expressed as carotene. Carotene concentrations are corrected for moisture losses on irradiation and are expressed uniformly on a basis of 13.5% moisture.

Flour No. 2197, with a carotene concentration of 2.06 p.p.m., was treated with dibenzoyl peroxide (Novadel reagent) at the rate of 22.68 gms. (0.8 oz.) per barrel of flour. Immediately after the 10 minutes mixing period the flour was spread on a board in a thin layer, approximately 1 mm. deep, and irradiated for definite time intervals exactly as described in the publication already referred to (Ferrari, Croze, and

Bailey, 1932). Without irradiation, the concentration of carotene fell in 24 hours to 0.64 p.p.m. It was possible to attain an equivalent bleaching effect (0.65 parts of carotene per million of flour) by irradiating the sample for 50 minutes.

When the same flour sample was treated with half the quantity of Novadel reagent, namely at the rate of 11.34 gms. (0.4 oz.) per barrel of flour, then in 24 hours the non-irradiated sample contained 1.03 parts of carotene per million of flour. Irradiation of the freshly treated sample for 30 minutes reduced the carotene to 1.08 p.p.m.

In order to examine the phenomenon when the flour had an initial bleaching treatment with nitrogen trichloride (Agene treatment), flour No. 2373 was treated first with Agene at the rate of 2 gms. per barrel, and its carotene concentration was reduced by this means to 1.14 p.p.m. This sample was then treated with dibenzoyl peroxide (Novadel reagent) at the rate of 22.68 gms. (0.8 oz.) per barrel of flour, and the carotene concentration of the non-irradiated sample fell in 24 hours to 0.48 p.p.m. Irradiation for 40 minutes produced the same bleaching effect as standing 24 hours, and the sample contained then 0.45 parts of carotene per million parts of flour. The same flour treated at the rate of 11.34 gms. (0.4 oz.) of Novadel reagent per barrel of flour had a carotene concentration of 0.58 p.p.m. after standing 24 hours, and irradiation of the treated sample for 30 minutes accomplished the same result, reducing the carotene concentration to 0.53 p.p.m.

Discussion

The results of the above experiments are summarized in Table I. A comparison of the results obtained with the quartz and the Uviol

TABLE I

BLEACHING ACCELERATION OF DIBENZOYL PEROXIDE (NOVADEL REAGENT) TREATED FLOURS ACCOMPLISHED BY A QUARTZ MERCURY VAPOR ARC
Results calculated to 13.5% moisture basis

Flour number	Unbleached flour—Carotene p.p.m.	Rate of Novadel bleaching treatment		Non-irradiated bleached flour after standing 24 hrs.—Carotene p.p.m.	Irradiated bleached flour	
		Gms./Bbl.	Oz./Bbl.		Irradiation time—Minutes	Carotene p.p.m.
2197	2.06	22.68	0.8	0.64	50	0.65
2197	2.06	11.34	0.4	1.03	30	1.08
2373	1.14	22.68	0.8	0.48	40	0.45
2373	1.14	11.34	0.4	0.58	30	0.53
2197	2.06	None	None		60	1.07
2373	1.14	None	None		60	0.63

glass mercury vapor arc is given in Table II, where the irradiation time necessary to produce equivalent bleaching acceleration with the two types of arc is given. The figures in Table II were arrived at, not only from a consideration of the data in this and the previous paper on this subject, but also from a study of unpublished data of great detail. The minutes of irradiation are those necessary to accelerate the bleaching action to a point where the concentration of carotene in the flour is the same as if the non-irradiated sample had stood for 24 hours.

TABLE II

A Comparison of the Effectiveness of a Quartz and a Uviol Glass Mercury Vapor Arc for Accelerating the Bleaching Action of Dibenzoyl Peroxide to the Level Reached by the Non-irradiated Sample on Standing 24 Hours

Carotene in unbleached flour—p.p.m.	Rate of Novadel bleaching treatment		Minutes of irradiation required for producing similar bleaching effect to standing for 24 hours	
	Gms./Bbl.	Oz./Bbl.	Quartz electrode	Uviol glass electrode
2.00±	22.68	0.8	50	60
2.00±	11.34	0.4	30	50
1.2 ±	22.68	0.8	40	100
1.2 ±	11.34	0.4	30	60

The data seem to justify the following statements: When the initial carotene concentration was in the range of 2 parts per million parts of flour and the sample was treated with dibenzoyl peroxide (Novadel reagent) at the rate of 22.68 gms. (0.8 oz.) per barrel of flour, it required 50 minutes irradiation with the quartz mercury vapor arc to achieve the bleaching effect obtained by standing 24 hours. The Uviol glass mercury vapor arc under the same conditions required 60 minutes. Upon treating the same flour with 11.34 gms. (0.4 oz.) of dibenzoyl peroxide (Novadel reagent) the irradiation times required to achieve the 24-hour bleaching effect with the quartz and Uviol glass electrodes were 30 and 50 minutes, respectively.

Similar comparisons, using a flour with substantially less carotene, say around 1.2 p.p.m., indicated that for a bleaching treatment with dibenzoyl peroxide (Novadel reagent) at the rate of 22.68 gms. (0.8 oz.) per barrel of flour, the time of irradiation with the quartz and Uviol glass electrode was 40 and 100 minutes, respectively. Using 11.34 gms. (0.4 oz.) of the Novadel reagent, the quartz and Uviol glass time ratios became 30 and 60 minutes, respectively. It is quite apparent that, under these conditions, the difference in efficiency between the two types of electrodes was considerable.

Bleaching Action of Ultra-Violet Radiant Energy Alone

A comparison of the bleaching action of the radiant energy alone from the quartz and Uviol glass mercury vapor arcs indicated again that a great difference existed in the actinic effect of the two arcs. The mercury arc in Uviol glass had little measurable effect on the concentration of carotinoid pigments in flour irradiated for 40 to 60 minutes. The mercury arc in quartz, however, bleached the flour markedly in 10 minutes. In 60 minutes flour No. 2197, with an initial carotene concentration of 2.06 p.p.m., was bleached to 1.07 p.p.m., and flour No. 2373 went from 1.14 to 0.63 p.p.m It is apparent that ultra-violet radiant energy of sufficient intensity and proper spectral energy distribution, such as is emitted by a quartz mercury vapor arc, has alone a decided bleaching effect on the carotinoid pigments of flour.

Color Comparisons Made With the Pekar Test

Having in mind that the bleaching accelerator would find more extended use in mill laboratories, were it feasible to make color comparisons of irradiated flour with the familiar Pekar test, experiments with dry and wet slicks were conducted. Exposures to irradiation with the quartz mercury vapor arc were made for periods varying from 10 to 60 minutes. In all cases the dry slick showed that the non-irradiated samples were distinctly lighter in color than the irradiated samples. As the irradiation time was increased the irradiated samples became progressively darker and the 60-minute treatment gave a slick that was a very dark, dirty brown color. The wet slick tests gave similar results but to a more marked degree, as did the dried wet slicks.

Measurements of temperature under the quartz electrode gave results from 24 to 54° C., depending on the irradiation time. The temperature reached a maximum in about 35 minutes and did not increase up to 60 minutes. The effect of heat alone on dibenzoyl peroxide treated flours has been investigated in this laboratory, and from our data it appears very improbable that the temperature, to which the irradiated flour is exposed, is responsible for the observed darkening. Discoloration of the slick was apparent after ten minutes irradiation when the temperature was only 44° C.

In an effort to learn whether ultra-violet radiant energy alone, or dibenzoyl peroxide, under the influence of ultra-violet radiant energy, was responsible for the observed darkening of the flour slicks, flours not treated with the Novadel reagent were irradiated. The same darkening occurred, indicating that the radiant energy was responsible and not the peroxide.

Moisture losses would influence the color of the Pekar test in the

manner described by Alcock and Ediger (1929), but such effects are overshadowed by the browning action of ultra-violet radiant energy. In fact, the latter effect is so pronounced as to make the Pekar test entirely unsatisfactory for determining the extent of color removal of irradiated flours.

Rotating Cylinder Transparent to Ultra-Violet Radiant Energy

A closed rotating cylinder, transparent to ultra-violet radiant energy, was constructed. Its purpose was (1) to conserve moisture; (2) to effect a quicker bleaching acceleration by continuously exposing fresh surfaces of flour to the actinic radiant energy; (3) to prevent overheating any part of the flour by keeping it agitated. The cylinder was constructed with walls of cellophane that transmitted ultra-violet energy with little diminished intensity down to 254 mμ (as far as the transmission was measured with a Bausch & Lomb quartz Monochromator). The cylinder had solid, wooden ends, and was mounted to rotate slowly under the quartz electrode. The device failed to effect a quicker bleach. In fact it did not bleach so efficiently as the sample spread on the board under the mercury arc. The reason probably is that fine flour dust accumulated on the inside cellophane surface and prevented the radiant energy from reaching the main body of flour at the bottom of the cylinder. Some modifications of this principle may possibly give better results.

Summary

Experiments for accelerating the bleaching action of dibenzoyl peroxide treated flours with radiant energy from a quartz mercury vapor arc are described and compared with similar experiments, in which the electrode vessel is constructed of Uviol glass. The actinic effect of the quartz mercury vapor arc is much greater.

The extent of bleaching is determined by measuring spectrophotometrically the transmittancy of flour extracts, and results are expressed for convenience as parts of carotene per million parts of flour. The time of irradiation required to accelerate the bleaching action to the point reached by the non-irradiated flour after standing 24 hours forms the basis of the comparisons. In addition to the nature of the electrode, two other factors determine the time required for irradiation. They are the initial concentration of carotene in the unbleached flour and the quantity of Novadel reagent used. For equivalent dosages of Novadel the lower carotene concentrations in flour require relatively longer irradiation times and the more Novadel reagent used the longer the irradiation time necessary to duplicate the bleaching action achieved on standing for 24 hours. Thus, samples containing approximately 2

and 1.2 parts of carotene per million parts of flour when treated with the Novadel reagent at the rate of 22.7 gms. (0.8 oz.) per barrel of flour required 50 and 40 minutes, respectively, irradiation treatment with the quartz mercury vapor lamp to bring the carotene concentration down to the level reached by standing 24 hours. When half the quantity of Novadel reagent was applied, namely: 11.3 gms. (0.4 oz.) per barrel of flour, 30 minutes of irradiation were required to achieve the requisite bleaching for both the flour containing 2 and 1.2 parts of carotene per million of flour.

Radiant energy alone from the quartz mercury vapor arc proved to be an effective bleaching agent. This was not true of the arc in Uviol glass.

The Pekar test was entirely unsatisfactory when used in connection with the bleaching accelerator for determining the extent of color removal, due to the discoloration of the sample by ultra-violet radiant energy.

In an effort to minimize moisture losses and excessive heating of the sample, as well as to possibly accelerate the bleaching effect of the quartz mercury vapor arc, a rotating cylinder with cellophane walls transparent to ultra violet radiant energy was used under the arc but without success, due probably to the screening effect of fine flour dust that adhered to the walls of the cylinder.

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A FLOUR EXTRACT AS A STANDARD IN THE COLORIMETRIC ESTIMATION OF FLOUR COLOR

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(Presented at the Annual Meeting, June, 1934)

The determination of flour color has been attempted in various ways. Most efforts have been directed toward measuring the color of a gasoline extract, and the difficulty has been chiefly finding a standard of comparison with the same hue as the flour extract. The following standards have been tried: a 0.25% solution of alizarin in chloroform; a 0.2% aqueous solution of potassium dichromate; a 0.005% aqueous solution of potassium chromate; a solution of potassium chromate in buffer solutions containing disodium hydrogen phosphate and potassium dihydrogen phosphate; potassium dichromate to which some cobalt nitrate is added; an aqueous solution of naphthal yellow and orange G; azobenzene in 96% alcohol; and probably others that have escaped our immediate attention. As a matter of fact, none of these standards has met with general favor, judging by the extent to which any one is used by cereal chemists. The chief reason is the difficulty experienced in matching the standards and the flour extract. Geddes, Binnington, and Whiteside (1934) have given recently a discussion of this problem. These authors demonstrated the marked superiority of a mercury vapor light source compared to daylight or its equivalent for matching the flour extract against potassium chromate standards. Using a very large number of determinations they computed a regression equation relating carotene concentration to gasoline color values, and it is now possible to compute by means of this equation the carotene equivalents corresponding to any determined gasoline color value. Such a procedure constituted an important advance in the determination of flour color by a colorimetric method, and lifted the gasoline color value determination from an unsatisfactory, arbitrary method of doubtful accuracy to a method in which the flour pigments may be expressed with adequate precision for routine work.

Arnaud in 1887 is credited by Palmer (1922) with first proposing a quantitative colorimetric method for estimating carotene in plant tissues. Arnaud used as a standard a 0.001% solution of carotene in carbon bisulphide. Willstätter and Stoll used standard carotinoid solutions containing 0.0134 gm. carotene in 0.5 liter of petroleum ether that

contains 5×10^{-5} moles per liter of alcohol. (Schertz and Merz, 1928.) The solution was stored in a well stoppered flask in the dark, and the authors state " in the course of three weeks it loses none of its intensity." The statement has been made by many investigators that carotene is not a suitable standard. This is undoubtedly true if this criticism is based on the assumption that pure, unoxidized, crystalline carotinoids are necessary for the preparation of the standards. It should be borne in mind, however, that crystalline material is not essential. The quantitative estimation of the carotinoids in solution can be accomplished by a spectrophotometric procedure. The only question regarding the suitability of such solutions as standards concerns their permanency of color.

A cleaner's naphtha-ethyl alcohol extract of flour does not constitute a solution solely of carotene. Other carotinoid pigments that may be present are extracted as well as fats, etc. Unpublished spectrophotometric work by the senior author has definitely shown the presence of pigments other than carotene in bran extracts. Chlorophyll and its decomposition products were indicated. The extent to which other substances interfere depends on the wheat product extracted, and patent flours are affected probably to a negligible degree. Neverthless there may be an advantage in using a flour extract itself as the colorimetric standard, because its spectral distribution curve and consequently its color attributes would correspond with those of the sample.

A cleaner's naphtha-alcohol extraction of a patent flour was prepared. To shield it from light it was stored in a brown, glass stoppered bottle on the laboratory shelf. The carotinoid pigments, conveniently expressed as carotene, were determined spectrophotometrically in the manner described by Ferrari (1933) at various intervals up to 291 days. The original concentration of carotene was 0.543 milligrams per liter corresponding to 2.71 parts per million of flour and the latter value was 2.63 at the end of 291 days. Results for intermediate times are given in Table I. A change in concentration corresponding to less than 0.1

TABLE I

CHANGE IN CAROTENE CONCENTRATION OF STANDARD FLOUR EXTRACT OVER A PERIOD OF TIME

Age of a flour extract standard Days	Carotene, mg/L	Equivalent parts of carotene per million parts flour—based on conventional procedure
0	0.543	2.71
34	0.540	2.70
98	0.554	2.77
175	0.538	2.69
230	0.524	2.62
291	0.526	2.63

part of carotene per million of flour over a period of 291 days is not considered significant, and the use of this extract as a colorimetric standard thus appears to be satisfactory, as this particular standard had satisfactory keeping qualities from the color standpoint for at least nine months. It is suggested that the standard flour extract be prepared by a laboratory having facilities for determining the concentration of carotene spectrophotometrically and distributed to other laboratories for use colorimetrically either with a standard type Duboscq colorimeter or with Nessler tubes. Checking the extract at intervals would be desirable or new flour extracts could be furnished, say, every six months.

Using a Duboscq colorimeter a flour extract standard was used to determine the carotene concentration of a small series of flour samples. Unlike the chromate and dichromate standards the hues of the standard and the extract matched perfectly, using an ordinary artificial daylight light source. The only errors therefore encountered in matching the colors were those inherent in a colorimetric procedure of this type. Some typical results are given in Table II and a comparison made with

TABLE II

COMPARISON OF SPECTROPHOTOMETRIC AND COLORIMETRIC VALUES, FOR CAROTENE IN FLOUR

Sample	Spectrophotometer values	Colorimeter values Standard 2.70 p.p.m.	Δ	Colorimeter values [1] Standard 2.63 p.p.m.
A	0.69	0.65	0.04	0.63
B	1.11	1.12	0.01	1.09
C	1.48	1.43	0.05	1.39
D	3.67	3.75	0.08	3.65
E	0.73	0.82	0.09	0.80
F	1.05	1.11	0.06	1.08
G	1.49	1.47	0.02	1.43
H	1.95	1.98	0.03	1.93
J	2.60	2.71	0.11	2.64
K	2.96	2.99	0.03	2.91

[1] These values show the small variation due to the deterioration of the standard.

the results for carotene obtained by examining the same solutions spectrophotometrically. No special care was taken in obtaining the results and they are probably typical of what may be expected in routine determinations. The agreement between the spectrophotometric and colorimetric determinations are considered good enough for routine work. Geddes, Binnington, and Whiteside (1934) have pointed out that Nessler tubes containing even units of carotene standards are superior to a Duboscq colorimeter from the standpoint of both accuracy and the time involved in making determinations. By using a highly

pigmented unbleached flour and a suitable flour to solvent ratio a deeply pigmented extract can be obtained, and from it suitable dilutions made to use as standards in Nessler tubes.

It is of course possible to use solutions of carotene, and a concentrated preparation in oil is available on the market at reasonable cost.[1] The use of a flour extract itself, however, has certain advantages that already have been pointed out.

Summary

An extract of flour prepared by using as a solvent a mixture of 93 parts of cleaner's naphtha and 7 parts of absolute ethyl alcohol was stored in a brown, glass stoppered bottle on the laboratory shelf. It had an original concentration of 0.543 milligrams of carotene per liter, corresponding to 2.71 parts of carotene per million parts of flour when expressed on the basis of the procedure used in flour color determinations in this laboratory. After 291 days the concentration of carotene expressed on the latter basis was 2.63 p.p.m. It thus appears that a flour extract itself prepared and stored as described constitutes a satisfactory standard for colorimetric comparisons. It has the advantage over all standards thus far proposed of being identical in color properties to the sample being measured. Tests confirmed its usefulness as a standard for determining the color of flour extracts using a Duboscq colorimeter. It appears feasible to prepare a flour extract with a satisfactory high carotene concentration, to determine its carotene concentration spectrophotometrically, and to use this extract as a colorimetric standard for expressing flour color conveniently as carotene.

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FACTORS AFFECTING THE DIASTATIC ACTIVITY OF WHEAT FLOUR[1]

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(Presented at the Annual Meeting, June, 1934)

Much attention has been given to diastatic activity of flour during the past few years, but most of this has been concerned with analysis, control, and effects upon baking tests. Relatively little attention has been paid to the underlying causes of variable diastatic activity. Mangels (1926) has demonstrated varietal influences, but more recently Landis (1934) surveyed the literature and came to the conclusion that all variations are environmental in nature. Much work has been done on increasing diastatic activity by reducing particle size. Kozmin (1933) has shown that the European wheat conditioning systems reduce diastatic activity. So with these scattering observations in mind an investigation of certain factors affecting this property of flours was undertaken.

The milling method was first studied, using seven spring wheats of the 1932 crop. Each lot was divided into 4 aliquots, and one aliquot of each was milled in an atmosphere of 50% relative humidity using a 10XX silk for separating the patent flour, one at 75% relative humidity using the 10XX silk, and the other two at the two humidity levels using the much finer 16XX cloth. Diastatic activity values for these flours are given in Table I.

TABLE I

EFFECT OF MILLING METHOD UPON DIASTATIC ACTIVITY OF PATENT FLOUR
(*Rumsey Units*)

Relative humidity of mill room atmosphere	50%	75%	50%	75%
Patent Bolter Silk	10XX	10XX	16XX	16XX
Sample No. 711	384	446	680	402
" 712	576	572	660	624
" 713	380	252	508	312
" 714	386	224	616	314
" 715	264	268	520	310
" 716	362	228	422	326
" 718	388	416	488	346
Mean	391.43	343.71	556.29	376.29

[1] Published with the approval of the Director as Paper No. 1276, Journal Series, Minnesota Agricultural Experiment Station.

When the flour was ground so as to pass the 16XX cloth at low humidity the diastatic activity was increased 42% over the diastatic activity of the flour ground to pass the 10XX silk, but when the milling was carried out at high humidity the increase was only 9%. The effect of increasing humidity when using the 10XX silk was to decrease the diastatic activity by 12%, and when using the 16XX silk the diastatic activity was reduced 32%. These results indicate that granulation alone was not the most important item since in the flours of finest granulation the 16XX high humidity flour was lower in diastatic activity than the 16XX low humidity flour. It is more probable that the condition of the outside of the starch granules was the major factor, because when the middlings were ground in a dry atmosphere the diastatic activity was greater than when they were ground in a humid atmosphere. This may not mean actually ruptured starch granules, but at least a modified surface due to dry grinding.

The next factor studied was the tempering of the wheat prior to milling. Bulk Thatcher (hard red spring) wheat of the 1933 crop was divided into 2000-gram portions and given different tempering treatments ranging from no temper to increasing the moisture content by exposure to humid air over a period of 7 to 10 days, and by different variations of the usual method of adding water to the wheat sufficient to bring it to 13.5% moisture, then placing in a closed can for a week, and then giving a second temper overnight before milling. The results of these types of treatment are shown in Table II and Figure 1. Loaves number 8 which were baked with varying sugar levels, 0, 2½%, and 6% were from flour which had received prior to milling, the usual tempering treatment involving wetting of the wheat to a 13.5% moisture level, storing in a closed can for a week, and giving a second temper of 2½% water the night before milling. The diastatic activity of this flour was 96 Rumsey units. Flours numbered 1 to 7 inclusive were similar to number 8. Loaves number 9 were from flour which was milled from wheat that was exposed to air of 75% relative humidity for a period of 10 days prior to milling. This wheat, which originally contained somewhat less than 10% of moisture, had gained nearly 4% in moisture. The diastatic activity of this flour was 186 or about double that of number 8. Flour number 10 was milled from untempered wheat. The diastatic activity of this flour was 170.

Winter wheat was tested in like manner and with similar results. The results with later tests of spring as well as the winter wheat are shown in Table III. It appears likely that much of the variability in diastatic activity of experimentally milled flours is occasioned before the flour is milled. At the Minnesota Experiment Station laboratories the tem-

TABLE II

EFFECT OF TEMPERING UPON DIASTATIC ACTIVITIES AND LOAF VOLUMES OF PATENT FLOURS
(Thatcher Wheat, 10% Moisture)

Run number	First temper					Second temper					Diastatic activity Rumsey units	Loaf volumes		
	Amount of water added	Time in days	Temperature	Container	Humidity	Amount of water	Time in hours	Temperature	Container	Humidity		No sugar	2½% sugar	6% sugar
	P.ct.		*° C.*			*P.ct.*		*° C.*				*Cc.*	*Cc.*	*Cc.*
1	3	10	25	Can	Closed	2½	16	30	Pan	95%	104	—	465	—
2	"	"	"	"	"	"	17	25	Can	Closed	112	—	530	—
3	"	"	"	"	"	"	18	25	Pan	40%	110	—	482	—
4	"	"	"	"	"	"	18	5	Can	Closed	94	—	467	—
5	"	"	5	"	"	"	19	25	Pan	40%	114	347	517	847
6	"	"	"	"	"	"	20	25	Can	Closed	105	350	492	850
7	"	"	"	"	"	"	21	30	Pan	95%	98	322	437	812
8	"	"	"	"	"	"	22	5	Can	Closed	96	325	450	767
9	0	11	25	Pan	75%	—	—	—	—	—	186	375	610	787
10	0	11	25	Pan	40%	—	—	—	—	—	170	337	560	705

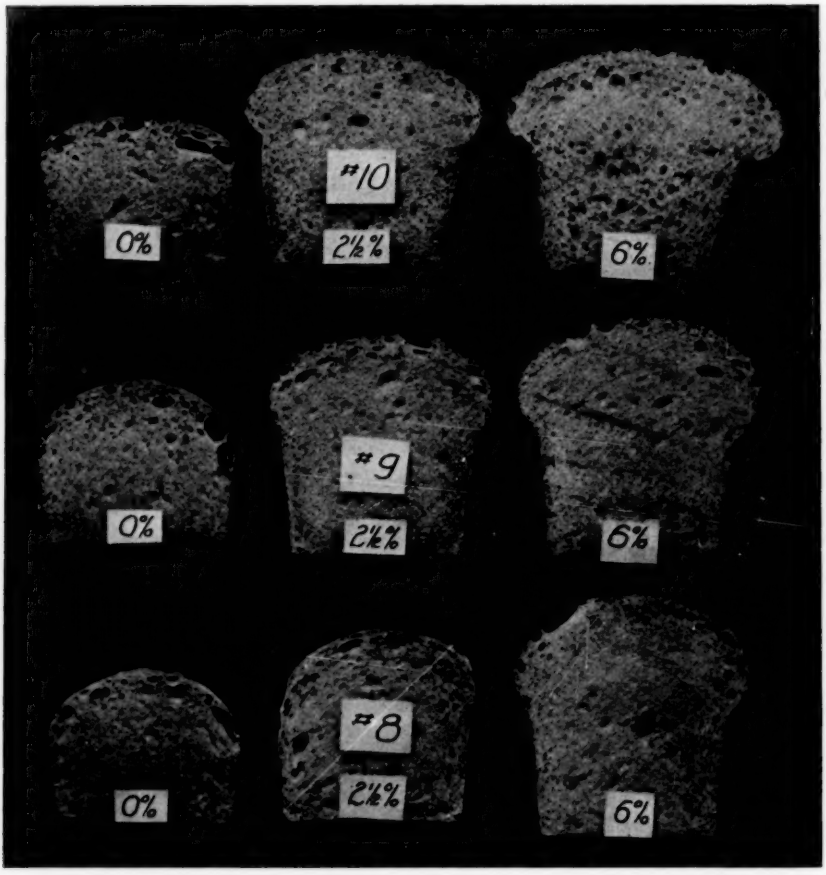

Fig. 1. Bread baked from flours having had varying tempering treatments prior to milling. Each flour baked with 0, 2½%, and 6% sugar in dough.
8—Tempered by means of liquid water.
9—Tempered by means of humid air.
#10—No tempering treatment.

pering method now in use, as a result of these investigations, is to expose the wheat in open pans to air of 75% relative humidity for 7 to 10 days, or until it reaches 13.5% moisture, and then add about 2½% liquid water an hour before milling.

The next stage of the investigation involved a comparison of the diastatic activity of flours produced in commercial and experimental milling. In this work 23 lots of wheat were milled both on the Pillsbury semi-commercial mill, and on the station experimental mill using 2000-gram samples. (The data are given in Table IV.) The correlation between the two milling methods in terms of diastatic activity of the flour was $r = .63$ which indicates a fairly good agreement. Diastatic

TABLE III

EFFECT OF TEMPERING UPON DIASTATIC ACTIVITIES OF FLOURS MILLED FROM THATCHER (HARD RED SPRING) AND MINTURKI (HARD WINTER) WHEATS (*Rumsey Units*)

First temper					Second temper		Diastatic activities			
							Minturki		Thatcher	
Amount of liquid water added	Time in days	Temperature	Container	Humidity	Time in hours	Amount of water added	Patent	Clear	Patent	Clear
P.ct.		° *C.*				*P.ct.*				
0	8	30	Pan	95%	None	—	94	157	183	210
0	8	25	Pan	70%	1	2½	98	126	150	192
5	8	25	Can	Closed	18	2½	100	92	128	130
5	alternately wet and dried 3 times				18	2½	94	134	125	153

TABLE IV

COMPARISON OF DIASTATIC ACTIVITIES OF COMMERCIALLY MILLED PATENT FLOUR, EXPERIMENTALLY MILLED PATENT FLOUR, AND FINELY GROUND WHOLE WHEAT MEAL (*Rumsey Units*)

Sample number	Variety	Origin	A Diastatic activity commercial flour	B Diastatic activity experimental flour	C Diastatic activity whole wheat meal
1924	Marquis	Fessenden, N. D.	226	140	260
1925	Ceres	" " "	278	202	270
1937	Reward	" " "	259	184	267
1928	Marquis	Leeds, N. D.	238	149	255
1929	Ceres	" " "	236	196	260
1943	Reward	" " "	284	153	245
1945	Marquis	Fargo, N. D.	230	148	287
1946	Ceres	" " "	300	204	317
1957	Reward	" " "	246	198	270
1959	Thatcher	" " "	279	172	290
1950	Marquis	Ft. Benton, Mont.	234	180	287
1951	Ceres	" " "	290[1]	204[1]	—
1953	Thatcher	" " "	234	140	287
1954	Supreme	" " "	224	165	255
1956	Comet	" " "	280	188	265
1938	Marquis	Crookston, Minn.	282	176	300
1941	Thatcher	" "	277	178	257
1968	Marquis	Montevideo, Minn.	283[1]	219[1]	—
1965	Thatcher	" "	226[1]	165[1]	—
1967	Marquis	Morris, Minn.	284[1]	183[1]	—
1942	Thatcher	" "	238	154	240
1940	Marquis	Waseca, "	204	140	270
1939	Thatcher	" "	201	142	252
	Mean		250.00	168.89	270.21
	Standard deviation		28.28	22.15	19.30

Correlations

$r_{AB} = +.63$ $r_{AC} = +.37$ $r_{BC} = +.33$
$n = 19$ 5% point = .43 1% point = .55

[1] Omitted from calculations because of incomplete data.

activities of the flour and of the finely ground whole wheat meal were correlated by the order of $r = .37$ for the commercial flour and $r = .33$ for the experimental flour.

In this series it was observed that certain environmental factors were at work since Marquis wheat from western Minnesota was higher in diastatic activity than Marquis wheat from Dakota or Montana. However, Ceres wheat was found to be higher in diastatic activity than Marquis grown at the same locations. Reward wheat was intermediate between Ceres and Marquis, while the variety Thatcher was about the same in diastatic activity as Marquis.

The effects of environment and variety were further studied in a series of 13 spring wheat varieties grown at 3 locations, and 7 winter wheat varieties grown at 2 locations in Minnesota in 1933. The individual values are given in Tables V and VI. In the spring wheat series

TABLE V

DIASTATIC ACTIVITIES OF EXPERIMENTALLY MILLED PATENT FLOUR AND GROUND WHOLE WHEAT MEAL FROM VARIETIES OF SPRING WHEATS GROWN AT THREE MINNESOTA STATIONS IN 1933. (*Rumsey units*)

Sample number	Variety	Univ. Farm	Waseca	Crookston	Univ. Farm	Waseca	Crookston	Mean of Stations	Mean of Stations
		Flour *A*	Flour *B*	Flour *C*	Wheat *D*	Wheat *E*	Wheat *F*	Flour *K*	Wheat *L*
2610	Double Cross	151	166	193	245	284	270	170	266
2308	Reliance	138	170	148	260	340	285	152	295
2223	Ceres	173	235	206	304	320	330	205	318
2297	Hope	180	164	167	296	313	263	170	291
2315	Double Cross	151	171	180	280	320	255	167	285
2340	" "	152	170	171	257	281	255	164	264
2621	" "	149	168	167	272	279	258	161	270
2607	" "	141	156	186	248	280	252	161	260
2303	Thatcher	142	168	212	261	302	288	174	284
2202	Marquillo	151	144	151	240	284	260	149	261
2309	Supreme	148	144	166	266	261	293	153	273
2204	Reward	162	155	170	271	288	255	162	271
1239	Marquis	131	157	162	254	281	288	150	274
	Mean	151.46	166.77	175.31	265.70	294.85	273.23	164.46	277.85
	Standard deviation	13.03	21.88	18.65	18.24	21.43	21.77	14.05	15.81

Correlations

$r_{AB} = +.43$	$r_{AD} = +.74$	$r_{DE} = +.49$	$r_{KL} = +.69$	$n = 13$
$r_{AC} = +.19$	$r_{BE} = +.54$	$r_{DF} = +.40$		5% point = .51
$r_{BC} = +.55$	$r_{CF} = +.36$	$r_{EF} = +.27$		1% point = .64

the interstation correlations for diastatic activity of experimental patent flour varied from $r = .19$ to $r = .55$. An inspection of the data showed Ceres wheat to be in the high diastatic group at all stations, while

TABLE VI

DIASTATIC ACTIVITIES OF EXPERIMENTALLY MILLED PATENT FLOUR AND GROUND WHOLE WHEAT MEAL FROM VARIETIES OF WINTER WHEATS GROWN AT TWO MINNESOTA STATIONS IN 1933. (*Rumsey units*)

Variety number	Univ. Farm	Waseca	Univ. Farm	Waseca
	Flour *G*	Flour *H*	Wheat *I*	Wheat *J*
2313	232	131	391	246
2614	188	258	340	178
2615	190	88	312	188
2616	125	141	235	230
2618	116	183	300	222
2551	146	146	290	208
1507	149	118	330	212
Mean	163.71	152.14	314.00	212.00
Standard deviation	38.26	50.78	44.53	21.80
	Correlations			
$r_{GH} = -.07$	$r_{GI} = +.82$		$n = 7$	
$r_{IJ} = +.05$	$r_{HJ} = -.34$		5% point = .67	

Marquis, Marquillo, Supreme and Reliance were always in the low diastatic group. Some varieties were either intermediate or variable in diastatic activity. In the winter wheat group there was no correlation between the two stations in diastatic activity of the flour.

When the diastatic activity values for spring wheat varieties from the 3 stations were averaged Ceres wheat was significantly higher than any other variety. Marquis, Marquillo, Supreme, and Reliance were in a low group, while Hope, Reward, and the Minnesota Double Cross Hybrids, including Thatcher, were intermediate in diastatic activity. To determine the relative diastatic activity of wheat varieties it was necessary to grow these wheats in comparable plots under different environmental conditions. Data obtained over a period of several years would be much more reliable than that from a single crop.

Diastatic activity determinations were also made upon finely ground samples of the wheats. The correlation between diastatic activity of wheat and that of flour for spring wheats at each of the three stations varied from $r = .36$ to $r = .74$, and for winter wheats over a wider range. When all 53 comparisons of wheat and flour were thrown into a single correlation the coefficient was $r = .36$ which is similar to that obtained in the earlier series. However, when mean values for wheat and flour diastatic activities of each variety were correlated, the correlation was $r = .69$. This indicated that if the varieties of wheat were grown in several locations and mean results were used it would be possible with a fair degree of accuracy to predict the relative diastatic activity of varieties for commercial production.

Summary

Particle size in itself was not found to be a major factor in determining the diastatic activity of a flour.

Condition of the surface of the flour particles as affected by the humidity of the air during the grinding and bolting of the middlings was found to be of great importance in determining the diastatic activity of a flour.

Method of tempering or conditioning the wheat prior to milling in a small experimental mill was found to influence the diastatic activity of the finished flour.

Diastatic activity of experimentally milled flour was found to be correlated with the diastatic activity of flour milled in a commercial flour mill from aliquots of the same wheat lots.

Environmental factors during the growing period of the wheat were found to affect the diastatic activity of the flour milled from the wheat.

Varietal differences in diastatic activity of flours milled from different varieties of wheat were noted.

Replication of variety tests in different locations was found to be essential in determining varietal differences in diastatic activity.

Prediction of the relative diastatic activities of flours milled from different wheat varieties from determinations of diastatic activity made upon small wheat samples was found to be possible with a fair degree of accuracy if sufficient replications of the varieties were grown under different environmental conditions.

Acknowledgments

The authors wish to express their appreciation to the Civil Works Administration for the grant of funds under which part of this work was carried out, and to Elmer D. Johnson who made the numerous diastatic activity determinations used in this study.

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THE DIASTATIC ACTIVITY OF WHOLE WHEAT AND SOME OTHER CEREAL GRAINS AS DETERMINED BY THE BLISH-SANDSTEDT METHOD

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(Presented at the Annual Meeting, June, 1934)

Introduction

A knowledge of the diastatic activity of wheat flour as well as a knowledge of the diastatic activity of certain supplements for use in producing diastatically balanced flours by the miller has become outstandingly important.

At the present time, information with respect to the diastatic activity of the raw material—wheat, etc.—is not obtained, generally speaking, before processing, but afterwards. It is believed, and it has been earlier suggested by Blish and Sandstedt (1933), that a worthwhile contribution to the cereal chemistry literature would be made if a method or methods could be developed which would indicate in a fairly comprehensive way the probable diastatic activity of the raw material. The recent observations of Bohn and Machon (1933) with respect to the desirability of adequate diastatic activity of whole wheat flour supports this belief.

It is appreciated that any method which is developed for the determination of the diastatic activity of whole grain products must be strictly of the empirical type; that uniformity in fineness of grinding must be maintained because the maltose values obtained in the usual diastatic activity determination are directly proportional to the degree of fineness to which the sample is ground. While this condition admittedly is somewhat difficult to standardize and some variations in granulation are bound to occur, it is felt that errors introduced by this condition are of less importance than those inherent to a diastatic determination made on experimentally milled flours where uniform flour granulation is even more difficult to control.

It is further appreciated that in developing a method for the determination of diastatic activity of whole grain products that certain objections can be raised on the grounds that in determining the diastatic activity of wheat meal, for instance, the activity of certain types of diastatic enzymes not always normal to flour (at least in relative pro-

[1] Grain Division, Bureau of Agricultural Economics.
[2] Division of Cereal Crops and Diseases, Bureau of Plant Industry.
[3] Grain Division, Bureau of Agricultural Economics.

portions) is determined. Even so, it is again believed that this factor is of no greater importance than would be the variance in the diastatic activity of flour, if it were processed under different conditions.

While this investigation was directed, in the main, to the development of a method for ascertaining the diastatic activity of wheat, the application of the method for determining the diastatic activity of several other cereal grains was also studied.

Experimental

Materials

Approximately 200 samples of the 1933 wheat crop, selected to obtain regional as well as class differences, were used in this study. In addition there are included the results of tests made on a number of samples of corn, barley, and rye.

Methods

General: The method finally adopted for preparing the sample for analysis was agreed upon only after extensive trials had been made to insure reproducibility of results. Such factors as *fineness of division, moisture content of the sample, development of heat during grinding, drying of moist samples before grinding*—all had to be standardized before the method would reproduce itself.

It was found after many grinding trials with the equipment later to be described, that the hard wheats—hard red spring, durum, and hard red winter wheat—could be satisfactorily ground with moisture contents up to 13%; whereas with the softer wheats the upper limit is about 0.5% below this. See Table I.

The development of heat during grinding is, of course, important and should be avoided. Under the conditions herein described, temperature of grinding did not exceed 110° F. Careful attention to the technic of grinding will prevent rises in temperature higher than this. A gradual reduction process accomplished by passing the material through the burrs at least three times, the burrs being set at successively closer spacings guarantees against high and rapid rises in sample temperature.

Samples containing moisture in excess of 13% in the instance of the hard wheats, and 12.5% for the soft wheats, have to be carefully dried before they can be suitably ground. However, continued drying so that undue drying takes place (8% or 9%) should be avoided because under such conditions low diastatic activity values usually obtain. Grain drying temperatures not to exceed 100° to 110° F. for periods longer than 4 or 5 hours should prevail.

TABLE I

EFFECT OF MOISTURE CONTENT OF SAMPLE AND GRINDING ON THE DIASTATIC ACTIVITY OF WHEAT[1]

Hard red spring wheat					Soft red winter wheat				
Moisture content before grinding	Total diastatic activity	Blank	Diastatic activity Net	Diastatic activity 15 per cent moisture basis	Moisture content before grinding	Total diastatic activity	Blank	Diastatic activity Net	Diastatic activity 15 per cent moisture basis
P.ct.	*Mgs. maltose*	*Mgs. maltose*	*Mgs. maltose*	*Mgs. maltose*	*P.ct.*	*Mgs. maltose*	*Mgs. maltose*	*Mgs. maltose*	*Mgs. maltose*
11.30	224	56	168	159	11.00	240	56	186	176
11.50	225	56	169	161	11.80	236	56	182	173
11.80	225	56	169	161	12.10	246	56	192	185
12.20	230	56	174	167	12.50	230	56	176	171
12.40	220	56	164	158	12.70	214	56	160	154
12.73	230	56	174	167	13.00	232	56	178	172
12.90	230	56	174	167					

[1] Milligrams of maltose from 10 gms. of wheat-meal after 1 hr. diastasis at 86° F.

Due to the heat of friction generated during grinding, it is, of course, necessary to be sure that the ground meal is not of a higher temperature than the reagents or incubation chamber.

The grinder must be cleaned and frequently cooled between successive runs.

Grinding Equipment

A new Hobart coffee mill, Model 2929, using the pulverizing burrs set at points 3, 2, and 1, respectively, was the apparatus with which the samples of barley, rye, and wheat were ground. For corn the first break was made on the granulating burrs, finishing on the pulverizing burrs.

Details of method: Dry the sample so that its moisture content does not exceed 13% in the instance of hard wheats, 12.5% in soft wheats and rye, 12% for barley and corn. Weigh out 40 to 50 gms. of grain and reduce gradually (in three or more successive stages) to the finest possible state of subdivision permitted by the grinder. Temperature of grinding should not exceed 110° F. After cooling the meal to room temperature, weigh out 5 gms. of meal into a 125 cc. Erlenmeyer flask and proceed with the determination of diastatic activity by the method described by Blish and Sandstedt (1933). A 5% error in duplicate

tests, when using different portions of the same sample, can be expected. Determine the moisture content of the meal and report results on a uniform moisture basis.

By means of the method just described, the diastatic activity of four commercial classes of wheat was studied. The data accumulated are recorded in Tables II and III. Table II records the average diastatic activity value for each class, while Table III is a frequency table showing the variability in diastatic activity by class and area of production.

TABLE II

AVERAGE DIASTATIC ACTIVITY OF FOUR COMMERCIAL CLASSES OF WHEAT

Class of wheat	Number of samples	Average diastatic activity—15 per cent moisture basis
		Mgs. maltose [1]
Hard red winter	104	168
Soft red winter	33	156
Hard red spring	33	227
Durum	25	234

[1] Milligrams of maltose from 10 gms. of wheat-meal after 1 hr. diastasis at 86° F.

The figures given in the tables are, of course, for diastatic activity only and do not include the blank determination. If these values were included, some fifty points would have to be added to the average figures recorded in Tables II and III. The blank figures within any given class vary by approximately 15 points.

On the basis of averages, it was found that the spring grown wheats —the hard red spring and durum wheats—were highest in diastatic activity, while the winter grown wheats—the hard red winter as well as the soft red winters—were considerably lower in this characteristic, the soft red winter wheats exhibiting the lowest average enzymic activity of the four classes of wheat studied.

However, if occasion is taken to read Table III it will be found that a group of hard red winter wheat samples of Montana origin raised the general average for this class of wheat and that the majority of the hard red winter wheat samples examined had a lower diastatic activity than the soft red winter wheats. Indeed most of the wheats studied of low diastatic activity were of the hard red winter class.

The variability in diastatic activity within any given class, however, was very great. Within the hard red winter wheats studied the range was almost 200%. The range in the soft red winter wheats was approximately 90%; and in the spring wheats 100%. This appears to be a sound argument for the desirability of a diastatic activity test on the whole wheat before processing.

TABLE III

DIASTATIC ACTIVITY OF VARIOUS COMMERCIAL CLASSES OF WHEAT AS DETERMINED BY THE BLISH AND SANDSTEDT METHOD[1]

Source	Number of samples	Average diastatic activity 15 per cent moisture basis	Range in diastatic activity—Milligrams of maltose per 10 gms. of wheat-meal after 1 hr. diastasis at 86° F. Number of samples found to have the indicated activity in milligrams of maltose																				
			100 to 109	110 to 119	120 to 129	130 to 139	140 to 149	150 to 159	160 to 169	170 to 179	180 to 189	190 to 199	200 to 209	210 to 219	220 to 229	230 to 239	240 to 249	250 to 259	260 to 269	270 to 279	280 to 289	290 to 299	300 and over
Hard Red Winter Wheat																							
Chicago	10	148					2	3	3	1	1												
Enid	7	148		1	1	1	2		1				1										
Fort Worth	18	144		1	2	4	7	2		1	1												
Great Falls	14	205							1	1	1	3	3	1	2	1		1					
Indianapolis	6	141		1		1	2	2															
Kansas City	26	161	1	1	3	5	3	3		3		1	1	1	1	3							
Omaha	16	215				1	1	1		1		1	1	1	5			1		1	1	1	
Wichita	7	140			1	3	1	2															
Totals	104	168	1	4	7	15	18	13	5	7	3	5	6	3	8	4	0	2	0	1	1	1	0

TABLE III—(*Continued*)

Source	Number of samples	Average diastatic activity 15 per cent moisture basis	Range in diastatic activity—Milligrams of maltose per 10 gms. of wheat-meal after 1 hr. diastasis at 86° F.																					
			Number of samples found to have the indicated activity in milligrams of maltose																					
			100 to 109	110 to 119	120 to 129	130 to 139	140 to 149	150 to 159	160 to 169	170 to 179	180 to 189	190 to 199	200 to 209	210 to 219	220 to 229	230 to 239	240 to 249	250 to 259	260 to 269	270 to 279	280 to 289	290 to 299	300 and over
Soft Red Winter Wheat																								
Baltimore	6	167							4	2														
Cincinnati	5	169						1	2	1	1													
Indianapolis	5	137				5																		
Louisville	4	133		1				1			1		1											
St. Louis	5	150				2	1		1	1														
Toledo	8	149			2		1	2	2	1														
Totals	33	156		1	2	7	2	4	9	5	2	0	1											
Hard Red Spring Wheat																								
Great Falls	6											1	1	1										
Minneapolis	27									5	3	3	1	3	1	3	2	2	1	1	1	1	3	
Totals	33									5	3	4	2	4	1	3	2	2	1	1	1	1	3	
Durum Wheat																								
Minneapolis	25									1			5	2	4	3	2	3	2	1	2			

[1] Cereal Chemistry **10:** 189–202.

The findings with respect to the relative diastatic activity of the various classes of wheats is in but partial agreement with the earlier data published by Mangels (1926) who concluded from his experiments that spring wheats were relatively low in diastatic activity, and his data indicate a high diastatic activity for Kubanka durum wheat as compared with several varieties of common spring wheat. In the study reported herein the common spring wheats were found to be not only relatively high in diastatic activity as compared with the winter wheats but also that they do not differ materially from the durums in this respect. A brief study was also made of varietal differences in diastatic activity. The pertinent data are presented in Table IV. While the data are not

TABLE IV

DIASTATIC ACTIVITY OF 8 WHEAT VARIETIES GROWN UNDER IDENTICAL SOIL AND CLIMATIC CONDITIONS

Varieties	Diastatic activity			
	Total	Blank	Net	15 per cent moisture basis
	Mgs. maltose	*Mgs. maltose*	*Mgs. maltose*	*Mgs. maltose*
Minturki	236	38	198	188
Iowin	254	46	208	198
Turkey	230	36	194	184
Blackhull	260	42	218	208
Tenmarq	244	38	206	196
Kawvale	274	42	232	219
Quivera	270	46	224	213
Cheyenne	258	38	220	209

extensive, they do indicate that different varieties grown on the same soil series under the same climatic conditions do not differ in diastatic activity by more than 10%.

No difficulty was experienced with the Blish and Sandstedt method as laid down for wheat flour or wheat-meal of normal diastatic activity. With sprouted wheats or materials with diastatic activities of a higher maltose value than 300, it was necessary to change the amounts of $K_3Fe(CN)_6$ used. The other reagents, however, remained the same. Accompanying this modification, a 5 cc. aliquot of the diastatic extract was used instead of the 2 cc. aliquot as recommended by Blish and Sandstedt for wheat flour. This modification appeared necessary because (1) of the large sampling error which prevails incident to the use of a 2 cc. aliquot, and (2) because of the uncertainty as to whether sufficient reagent is present for reduction purposes when aliquots of high diastatic activity are being tested.

The magnitude of these errors is illustrated by the figures shown below in Table V.

TABLE V

DIASTATIC ACTIVITY OF CERTAIN WHEAT SAMPLES AS DETERMINED BY USING 2 CC. ALIQUOT AND 10 CC. OF $K_3Fe(CN)_6$ AS COMPARED WITH 5 CC. ALIQUOT AND 15 CC. OF $K_3Fe(CN)_6$

Sample number	Maltose value using a 2 cc. aliquot and 10 cc. of $K_3Fe(CN)_6$	Maltose value using a 5 cc. aliquot and 15 cc. of $K_3Fe(CN)_6$
1	330	348
2	312	344
3	355	388
4	390	412
5	360	364
6	300	360
7	430	436
8	330	392
9	485	524
10	436	428

Diastatic Activity of Rye

The application of the method above described for wheat works equally well on rye. The diastatic activity of rye is rather high. Converted to a 15% moisture basis the average diastatic activity (maltose value was 233) with a range of 93 points from high to low.

Diastatic Activity of Barley

A determination of the diastatic activity of ground barley by the aforementioned method offers some difficulties. During the incubation period the matrix acquires a gummy or gelatinous consistency making filtering very difficult; so much so that it is difficult to acquire even a 2 cc. aliquot within the 20-minute interval allowed before the reduction by ferricyanide must take place. This condition is not improved by the presence of the sodium tungstate in the medium.

Although rapid filtering through Gooch or Buchner filters was not tried, it is believed that such a procedure would be materially beneficial to the successful carrying out of the test. In spite of the admitted drawbacks to the test as applied to barley, a series of interesting maltose values were obtained that indicate a rather wide range in the diastatic activity of various barleys. Of the maltose values obtained, all exceeded 175.

Diastatic Activity of Corn

The greatest difficulty with this determination on corn is in the preparation of the sample. Considerable care has to be exercised to secure

uniform granulation without the development of heat. The oily nature of corn meal makes this procedure rather meticulous. The diastatic values found for corn are very similar to those found in this study for hard red winter wheat.

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DIASTATIC ACTIVITY IN SUSPENSIONS AND DOUGHS[1]

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(Presented at the Annual Meeting, June, 1934)

The Pioneer Section of the A. A. C. C. has for the past year carried on a collaborative checking program with respect to the determination of diastatic activity by the ferricyanide method (Blish and Sandstedt, 1933). In this connection they have accumulated some excellent data on the reliability of the ferricyanide method using ten different flours under widely varying conditions. The Pioneer Section has very kindly allowed the use of these data for the basis of this paper.

Discussion of Data

The diastatic activity of each flour as determined by each collaborator, and for each flour the standard deviation (σ), the probable error of a single determination (Es), and the probable error of the mean (Em) are given in Table I. The collaborators are arranged in the order of their increasing deviation from the average, *i.e.*, in the order of the decreasing reliability of their determinations. Table II gives for each collaborator his deviations from the averages, his standard deviation, and his probable error of a single determination.

The wide range in the probable error of a single determination for individual collaborators (Table II) indicates that we have a miscellaneous group of collaborators, some of whom are more reliable than others, either in their control of environmental conditions or in their strict adherence to specifications. In some cases systematic errors have been traced to inaccuracies of volumetric glassware, especially pipettes. The latter should always be carefully checked. If we eliminate Collaborators 16 and 17, who manifestly have either a faulty technique or improper control of conditions, and such erratic results as those of Collaborator 9 on flour number 4, Collaborator 13 on flour number 3, and Collaborator 15 on flour number 9, we find that the ferricyanide method does give excellent checks between laboratories. The very close checks between collaborators and the very low probable errors for the collabora-

[1] Subcommittee report, 1933–34 Committee on Methods of Analysis.

TABLE I

RESULTS OF COLLABORATIVE DIASTATIC ACTIVITY DETERMINATIONS

Collaborator	Flour									
	1	2	3	4	5	6	7	8	9	10
1	354	—	—	322	219	173	265	158	—	228
2	—	—	—	—	—	—	—	150	320	226
3	356	182	250	320	222	173	269	152	311	222
4	—	173	246	318	216	170	276	150	—	225
5	355	188	—	310	211	—	264	158	310	224
6	—	183	248	333	222	175	277	154	324	225
7	339	—	236	—	213	160	265	152	313	226
8	347	173	244	311	—	160	—	142	312	218
9	355	180	250	344	222	171	275	150	330	226
10	—	188	256	335	218	—	254	166	328	228
11	357	189	261	333	220	174	278	160	335	240
12	—	—	—	—	—	—	—	161	300	229
13	340	178	280	330	218	180	274	160	308	220
14	—	188	—	296	—	—	—	148	318	204
15	—	—	—	—	217	168	274	154	355	233
16	—	—	230	292	240	156	260	145	310	260
17	—	—	234	274	208	185	250	162	278	—
Mean	351	182	249	317	219	171	268	154	317	227
σ[1]	6.93	5.83	13.0	19.6	7.36	8.09	8.72	6.45	16.7	11.2
Es[2]	4.67	3.93	8.77	13.2	4.96	5.46	5.88	4.35	11.3	7.56
Em[3]	1.65	1.24	2.64	3.66	1.37	1.58	1.63	1.06	2.92	1.89

[1] Standard deviation.
[2] Probable error of a single determination.
[3] Probable error of the mean.

TABLE II

DEVIATIONS OF INDIVIDUAL COLLABORATORS FROM THE AVERAGES

Collaborator	Flour										σ[1]	Es[2]
	1	2	3	4	5	6	7	8	9	10		
1	+3	—	—	+5	0	+2	−3	+4	—	+1	2.93	1.98
2	—	—	—	—	—	—	—	−4	+3	−1	2.94	1.98
3	+5	0	+1	+3	+3	+2	+1	−2	−6	−5	3.38	2.28
4	—	−9	−3	+1	−3	−1	+8	−4	—	−2	4.81	3.24
5	+4	+6	—	−7	−8	—	−4	+4	−7	−3	5.65	3.81
6	—	+1	−1	+16	+3	+4	+9	0	+7	−2	6.81	4.59
7	−12	—	−13	—	−6	−11	−3	−2	−4	−1	7.91	5.33
8	−4	−9	−5	−6	—	−11	—	−12	−5	−9	8.13	5.47
9	+4	−2	+1	+27	+3	0	+7	−4	+13	−1	10.0	6.75
10	—	+6	+7	+18	−1	—	−14	+12	+11	+1	10.4	7.02
11	+6	+7	+12	+16	+1	+3	+10	+6	+18	+13	10.6	7.15
12	—	—	—	—	—	—	—	+7	−17	+2	10.7	7.22
13	−11	−4	+31	+13	−1	+9	+6	+6	−9	−7	12.5	8.44
14	—	+6	—	−21	—	—	—	−6	+1	−23	14.4	9.72
15	—	—	—	—	−2	−3	+6	0	+38	+6	16.0	10.8
16	—	—	−19	−25	+21	−15	−8	−9	−7	+33	19.2	12.9
17	—	—	−15	−43	−11	+14	−18	+8	−39	—	24.8	16.7

[1] Standard deviation.
[2] Probable error of a single determination.

tors in the upper part of Table II show that the method is reliable for practical control purposes.

Diastatic Activity in Doughs

The question is frequently raised as to whether the Rumsey type of method for estimating diastatic activity in flour-water suspension accurately reflects the diastasis that occurs in dough itself. It is difficult to obtain direct evidence on this point because the estimation of diastatic activity in a dough by methods involving the measurement of the maltose produced is not an entirely satisfactory procedure, due to the difficulty of completely disintegrating the dough so that one may be certain of complete extraction of the sugars. The dough may be disintegrated by the use of a power-driven egg beater as suggested by Landis (1933); or if an equal quantity of fine sand is mixed with the flour the dough can be quite readily disintegrated with a spatula.

Some comparative diastatic activity determinations were made, both in suspensions and in doughs, on flours of widely varying diastatic powers, by the ferricyanide method. The doughs gave values that were of the same order of magnitude and variation as the corresponding suspensions. However, with high diastatic flours there was a tendency for the doughs to give lower comparative values than the suspensions and this tendency increased with the diastatic activity of the flour, with increasing time of diastasis, and with decreasing absorption in the doughs.

This may possibly be explained by the greater accumulation of the end product (maltose) in the diastatically stronger flours, causing a slowing down of the reaction rate. This end product would be more highly concentrated in a dough than in a suspension consisting of one part flour to 10 of solvent. On this basis the Rumsey type of suspension method may be presumed to give values that are more informative than when the same procedure is applied to dough, for in actual baking, of course, the dough contains yeast which removes the end product of diastatic activity.

In order to test this theory, a series of flours was subjected to comparative tests by the ferricyanide method and by a manometric measurement of gassing power of small doughs containing 3% of yeast. The doughs were made with 10 gms. of flour, 0.3 gm. of yeast, and 7 cc. of water. The CO_2 pressure generated was measured in a special container fitted with a manometer as described by Sandstedt and Blish (1934). The pressure obtained after a 4-hour fermentation was converted to its sugar equivalent by dividing by .74—a factor obtained by the fermentation of pure sugars under the same conditions.

Table III gives the comparison between total fermentable sugars obtained by the dough fermentation method and the total sugars (maltose + sucrose) present in suspensions after 4 hours diastasis.

TABLE III

TOTAL SUGARS PRESENT IN SUSPENSIONS AFTER DIASTASIS AND TOTAL SUGARS FERMENTED FROM DOUGHS

Flour	Suspension 4-hour diastases			Dough 4-hour fermentation	
	Sucrose[1]	Maltose[1]	Total sugar[1]	Pressure[2]	Sugar equivalent[1]
2	101	533	634	468	633
5	117	423	540	405	547
13	116	258	374	277	374
18	100	277	377	306	413
26	110	252	362	280	378
28	125	260	385	299	404
32	140	349	489	363	491
38	84	136	220	167	226
39[3]	225	680	905	662	894
40	174	300	474	361	488

[1] Expressed as mgms. per 10 gms. of flour.
[2] Pressure in mm. of mercury.
[3] 5% of yeast was necessary with this flour because of its high diastatic activity.

There is satisfactory agreement between the results obtained by these two methods, showing that the Rumsey suspension type of procedure does reflect diastasis as it occurs in doughs containing yeast.

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CORRELATION BETWEEN DIASTATIC ACTIVITY AND GASSING POWER IN COMMERCIAL FLOURS[1]

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(Read at the Annual Meeting, June, 1934)

Rumsey (1922) established an autolytic procedure for evaluating the diastatic activity of a flour. In testing fourteen commercial flours it was found that the maltose produced served as a measure of the diastatic activity of the flour when in the form of a dough.

Karacsonya and Bailey (1930) found that overgrinding increased the Rumsey autolytic diastatic value, that the magnitude of the rise was in inverse ratio to the initial diastatic activity, and that overgrinding did not substantially change the gas production of the flour. They suggested that the Rumsey type of procedure at times may fail to measure fermentation potentialities as measured in terms of gas production.

Jørgensen (1931) is of the opinion that when the Rumsey autolytic procedure or one of its modifications is used, "gassing power" and diastatic activity of a flour are interchangeable expressions.

Elion (1932), in referring to the Rumsey autolytic type procedure for diastatic values of flour, states that the difference in conditions between this procedure and actual bread production should not be overlooked and that factors other than diastatic activity are influencing the question of whether and to what extent a shortage of sugar will occur during the rising of the dough; factors which are left out of account in the Rumsey type procedure.

Blish, Sandstedt, and Astleford (1932) regard diastatic activity and gassing power values of flour as not strictly synonymous terms because they are not a measure of identically the same thing. The former gives a measure of maltose formed, whereas the latter includes the original maltose and sucrose in the flour. The original maltose value of sound flours was found constant enough to be dispensed with or to be corrected for by a constant value. The original sucrose content in flours was found to vary considerably and therefore to have a direct bearing on the association of the two values.

Larmour, Geddes, and Whiteside (1933) graphically show a strik-

[1] Subcommittee report, 1933–34 Committee on Methods of Analysis.

ingly high positive correlation between diastatic activity and CO_2 production.

Because of the simplicity and accuracy of the improved Rumsey type procedure for the estimation of flour diastatic value (Blish and Sandstedt, 1933), and the simplicity of the manometric test for measuring gas production in flour suspensions (Blish, Sandstedt, and Astleford, 1932), and the numerous methods for measuring gas production from doughs, the writer has attempted to measure the association existing between values as determined by these methods on a relatively large group of commercial flours.

Diastatic Activity Measured in Flour Suspensions

The following data are based on two methods using a flour suspension, namely, the Rumsey type autolytic procedure and the manometric procedure as described by Blish, Sandstedt, and Astleford (1932) in which excess yeast is used.

Experimental

Material and Methods

Samples: One hundred twenty-two commercial flours were selected at random from bakeries which were supplied with flour from mills grinding spring and winter wheats. The ash range of these flours was from 0.37% to 0.70% with the major portion ashing 0.39% to 0.47%. The protein content ranged from 8.5% to 13.5% with the majority falling in the 11.00% to 12.5% class. One submitted sample was a cake flour and another was very abnormal in its gluten development process. These two flours were not used in the tabulated data except for special study.

Samples were received over a period of several months and represent wheats from two crop years.

Methods: In determining the diastatic value of these flours, the improved ferricyanide procedure described by Blish and Sandstedt (1933) was used with no blank values deducted. The averages of duplicate determinations were reported. The average standard error of a single determination was of the order $3.40 \pm .34$ mgms. maltose per 10 gms. of flour or 1.24% of the mean value of all flours studied.

The suspension gas pressure values were made using the equipment and procedure as described by Blish, Sandstedt, and Astleford (1932). Originally it was supposed that the excess yeast (300%) used in the described procedure would eliminate variations due to the metabolic activity of the yeast, but these variations were encountered and it was suggested that a correction factor be used to care for this variable fac-

tor. These tests were run in sets of 12, 6 duplicates with one flour as a standard for determining the yeast factor to correct the other tests. After a number of tests on the standard flour, the average value was established as a basic or arbitrary value for this sample. All reports were the average of duplicates and the average standard error of a single determination was 4.0 ± .42 mm. pressure or 2.14% of the mean value of all tests. This error was essentially the same for high and low diastase flours. The diastatic activity value of the standard flour remained constant within the limits of the test during the period that these data were compiled, while the gas pressure values ranged from 15 mm. above (minus factor) to 35 mm. below (plus factor) the basic value. The higher values were more common in the colder months and the lower values were associated with the warm season. This factor fluctuated considerably from day to day and even from morning to afternoon when yeast storage was poor. Regular morning deliveries of one pound

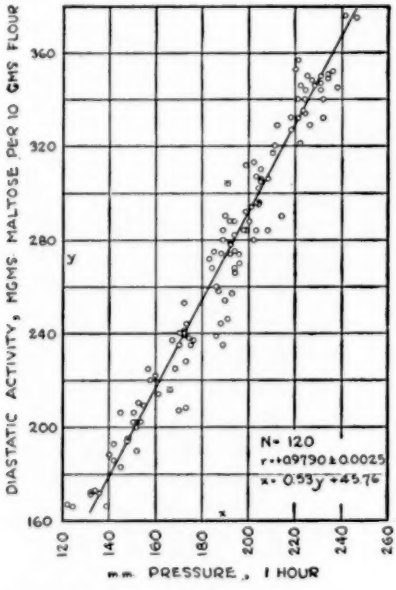

Fig. 1. Diastatic activity and Blish manometric suspension 1 hour values in commercial flours.

lots of bakers' yeast were used. Only one brand, yeast "A," was used except when a special study was made to determine the effect of other yeast types on the results.

When yeast "B" was used, the correction factor for the standard flour (using the basic value established with yeast "A") was minus 30 to 60 mm.; and when high or low gas producing flours were tested and properly corrected, the values checked very close to the values established when yeast "A" was used.

Figure 1 is a graphical representation of the data obtained on this group of samples. The range of diastatic values was from 166 to 376 mgms. with an average of 271.79 mgms. maltose per 10 gms. of flour. The gas pressure values ranged from 124 to 246 mm. with an average of 189.48 mm. pressure. The relationship is linear and can be reduced to the equation $X = .53Y + 45.76$ where $X =$ mm. pressure and $Y =$ mgms. maltose value per 10 gms. of flour. The correlation between the values of X and Y is expressed by the coefficient $+ .979 \pm .0025$. If X is to be used to predict Y then the prediction value is $1 - \sqrt{1 - r^2}$ or .796 for these data. The diastatic activity value predicted from the gas pressure value would vary from the determined diastatic activity value by a standard error of 11.25 mgms. maltose per 10 gms. of flour.

Discussion

It has been stated that "diastatic activity" and "gassing power" are not synonymous since the original sucrose content of flour is a variable factor which is measured by any gassing procedure which employs yeast. These data indicate that in flour suspensions the correlation between values established by the two methods with a random group of commercial flours is very high. From Figure 1 it will be observed, however, that there are several samples that fall some distance from the average line of relationship. This was true also of the two samples eliminated from the data. These samples were tested for their original gassing power by adding 5 cc. of N/10 HCl in 25 cc. water to 3 gms. of the flour, digesting 30 minutes to destroy all diastase, adding 5 cc. N/10 NaOH to exactly neutralize the acid, and proceeding with the gassing test in the regular manner (Blish *et al.*, 1932). It was found that between those flours showing relative high and low gassing ability as compared with the average in the regular test, there was as much as 21 mm. pressure difference due to the original fermentable sugars which is approximately equivalent to 40 mgms. maltose according to the established equation. The maltose blanks on these flours were essentially the same in all cases. This would indicate that the non-reducing sugar content of the flour was the major variable factor causing these instances of poor correlation. However, when sucrose and C. P. maltose were added to various flours and subjected to this one hour gassing procedure (with diastase destroyed), increasing amounts of sugar gave a linear increase in pressure readings, except that the rate of increase in pressure was not so great for maltose as it was for sucrose, due to differences in fermentation rate and the limited time of the test.

Additions of sucrose and C. P. maltose in increasing amounts to the regular manometric procedure gave a linear rise in pressure. When 1%

of these sugars was added separately to several flours, the average rise for sucrose was 58 to 60 mm. pressure, while for C. P. maltose it was 44 to 48 mm. When yeast " B " was used in these measurements, the rise was 68 to 70 mm. for both sugars, which indicated that yeast " B " possessed a different maltose-sucrose fermentation rate in the one hour period. The high pressure values for both sugars using yeast " B " was unexpected because " B " is a comparatively slow gas-producing yeast in the dough state.

When diastatic malt products were added in increasing amounts to flours not falling on this line of relationship of the two methods, the increased values of each test were essentially in proportion to the equation, or parallel to the line of relationship, but for this particular group of samples a new constant was necessary for the equation.

It is logical to expect the greater experimental error in the manometric procedure because presumably two biological materials are involved whereas only one biological material is involved in the Rumsey autolytic procedure. The magnitude of the error of a single determination can be accounted for in part because Erlenmeyer flasks are not uniform in volume and vary in the inside diameter of the necks, allowing some stoppers to go farther into the flask, thereby reducing the volume. Selected flasks were used in this work, but this still remained a source of error. The manometric tube diagrammed by Blish *et al.* (1932) is not of the proper range for some high gassing flours when a high minus yeast factor is encountered. This can be corrected by using 2½-gm. samples in such cases, and we found the following equation convenient for converting 2½-gm. sample results to 3-gm. sample values. When X' equals the 2½-gm. value then X (the 3-gm. value) equals $15 + 1.125X'$. It might be preferable to lengthen the manometric tube and bend it so that the mercury can be measured conveniently from level to level thereby minimizing the effect of uneven bore. The final gas pressure differs depending on whether the flasks are shaken continuously during the fermenting period, or are agitated lightly only occasionally during the one hour digestion. This must be considered when one uses this method to study rate of fermentation for periods longer than one hour.

In making determinations with prolonged digestion periods, best results may be obtained by starting as many tests as determinations are desired and agitating the flasks vigorously only when making the final reading. However, this involves difficulties in determining the proper yeast factor which can possibly be partly overcome by modifying the procedure to the use of 40% to 50% yeast (Landis, 1933) and minimizing the magnitude of the correction factor.

Diastatic Activity and Dough Gas-Production

The following data are based on the Rumsey autolytic type procedure and gas production from a dough similar to a bakeshop sponge.

Experimental

Material and Methods

Samples: Some of the flours used in the studies with flour suspensions just recorded were not available, other samples of similar description were added keeping the samples a random group of commercial flours.

Methods: Diastatic activity values were determined as previously described. Dough gas production values were determined by a procedure similar to that described by Heald (1932). One hundred grams of flour was mixed with 66 cc. of water and 2.5 gms. of yeast. This dough was fermented at 30° C. for 6 hours. The water displaced was measured every hour and the total 6 hour displacement was recorded. All tests were run in duplicate and the average reported. From day to day it was evident that yeast variations caused all tests to run high or low compared with previous tests, therefore a standard flour was run with each set of determinations and all values on that day were corrected by the amount this flour varied from its average 6-hour gas production. The average standard error of a single determination for this method was 18.03 ± 2.48 cc. per 100 gms. of flour when either high or low gassing flours were tested or 1.30% of the average of all samples tested.

Figure 2 is a graphical representation of the data obtained from this group of 120 flours by these two methods. The maltose values ranged from 166 to 423 mgms. with an average of 274.55 mgms. per 10 gms. of flour, while the gas-production values ranged from 820 cc. to 1898 cc. with an average of 1400.95 cc. per 100 gms. of flour. Apparently the relationship of the values tends to be non-linear. However, statistically it can be considered a straight line, and the association expressed by the correlation coefficient $+ .9271 \pm .0086$.

Discussion

The dough gas-production procedure used in establishing these results does not measure the maximum fermentation potentialities of a flour, because a dough punching schedule would give higher gassing values (Halton and Fisher, 1932). However, it does place all the flours on a comparative basis, for (using yeast "A") the 6-hour period carries them all through their peak of gas production to approximately a constant minimum rate.

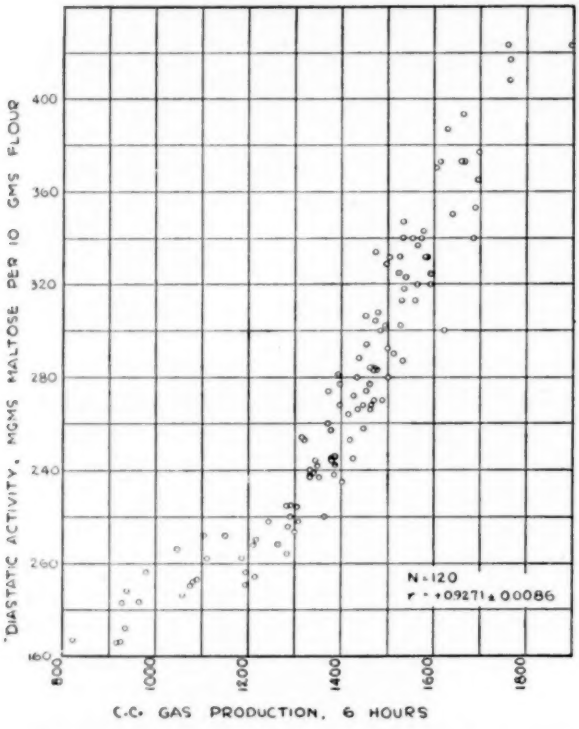

Fig. 2. Diastatic activity and total 6 hour dough gas-producton in commercial flours.

Using yeast "B," the fermentation rate differed, but the total gas produced was essentially the same on all low gassing flours when the results were corrected to the basic value of the standard flour established by the tests with yeast "A." Slightly low results from high gassing flours indicated that with this yeast the method should be modified either by adding more yeast or by extending the time.

Gas Production in Suspensions and Doughs

The gas pressure values for flour suspensions and the dough gas production values are represented graphically in Figure 3. For this series of 102 flours, the range of suspension gas pressure values was from 128 to 274 mm. (average 184.39 mm.), and the range of dough gas production values from 820 to 1898 cc. (average 1363.11 cc.) per 100 gms. of flour. The relationship again tends to be non-linear, but statistically can be considered linear and the association represented by the correlation coefficient $+ .9327 \pm .0087$.

In arriving at these values the same yeast was used in both methods

each day. While the yeast factor was evident for both methods, its magnitude was significant only in the case of the suspension procedure in which excess yeast was used. Little or no association between the factors for the two methods for the same yeast was detected.

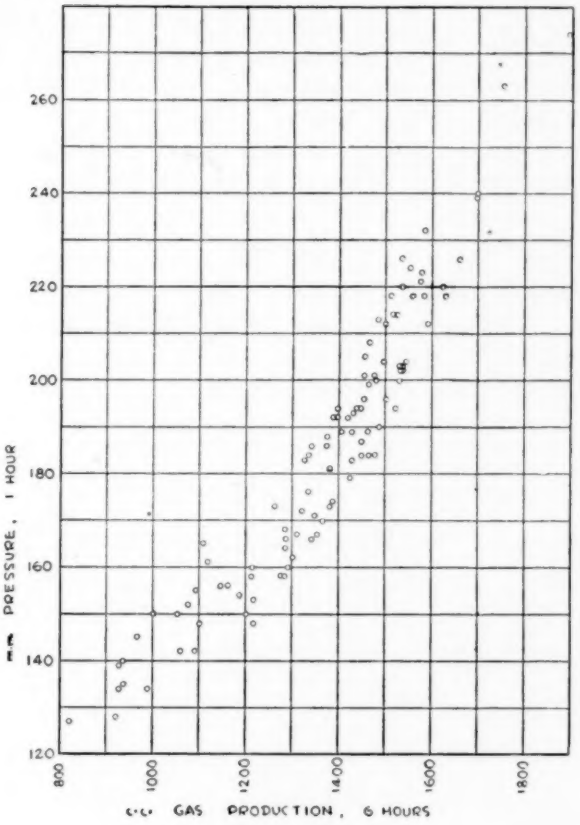

Fig. 3. Blish suspension manometric 1 hour values and total 6 hour dough gas-production in commercial flours.

Several flours having high suspension gas values, compared with the average relationship of the diastatic values, were tested in the dough gas-production procedure. On these, from 50 cc. to 150 cc. more gas was obtained than from flours of average suspension gas values and the same diastatic activity. This would indicate, as shown by Blish, Sandstedt, and Astleford (1932), that with sound flours of high or low non-reducing sugar content the gas suspension test is probably a better measure of dough gas-production than is the diastatic activity determination. The suspension type, gas-pressure procedure with

a yeast factor correction, also has its particular advantage over the dough gas-production procedure in that the reading can be taken at the end of an hour period and that the method can be modified to indicate the original sugar value of a flour.

The dough gas-production procedure has its disadvantage in the length of time required to complete the test. The advantages lie in that short interval readings (an automatic recorder may be used) give valuable information on the rate of gas production and the time at which the "critical point" is reached, as well as the effect on gas production of various dough batch ingredients. Also yeast variability is diminished by this procedure and the degree of accuracy can be made very acceptable.

Summary

In a random group of commercial flours, the degree of association was measured between the diastatic activity test, the Blish *et al.* manometric suspension test, and the total dough gas-production test. The numerical values established were as follows:

Method	Number of samples	Range of values	Mean value	Correlation coefficient	Standard error of single test	Standard error per cent of average of all values
Diastatic activity	120	166–376 mgs.	271.79 mgs.	+.9790 ±.0025	3.40 mgs. ± .34	1.24
Suspension gas-production 1 hr. excess yeast	120	124–246 mm.	189.48 mm.		4.00 mm. ± .42	2.14
Diastatic activity	120	166–423 mgs.	274.55 mgs.	+.9271 ±.0086		
Dough gas-production 6 hrs., 2.5% yeast	120	820–1898 cc.	1400.95 cc.		18.03 cc. ±2.48	1.30
Suspension gas-production 1 hr. excess yeast	102	128–274 mm.	184.39 mm.	+.9327 ±.0087		
Dough gas-production 6 hrs., 2.5% yeast	102	820–1898 cc.	1363.11 cc.			

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STUDIES ON THE VITALITY OF WHEAT. II. INFLUENCE OF MOISTURE IN WHEAT SEEDS UPON IMBIBITION AND SPEED OF GERMINATION

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(Received for publication March 27, 1934)

In passing from the dormant to the active state at proper temperatures, and in the presence of sufficient moisture, various seeds show differing capacities for absorbing water. As a rule, the amount of water absorbed is independent of the possession of life.

Duvel (1904) found, not unreasonably, that starchy seeds absorb or require more water than fatty seeds during germination, and a number of other workers have shown, as Shull (1911) with seeds of *Xanthium glabratum*, that the capacity of absorption of water during germination of seeds depends upon a number of variable factors.

Atkins (1909) showed that both living and dead seeds of *Phaseolus vulgaris* absorbed the same amount of water when immersed in a decinormal solution of sodium chloride, and concluded that the composition of the seed and its physical condition were more important factors in determining degree of imbibition than was the possession of life. This has been confirmed by us in the case of wheat, and we have shown further that the moisture-content of wheat before being set for germination is all important in determining speed of germination and in controlling the amount of necessary water, later absorbed, for bringing about that speedy germination.

We had found that normal air-dried, living English wheat, containing from 10% to 12% of moisture, absorbed 25% to 30% of water during germination in some 60 hours. Later, with some wheat artificially dried over calcium chloride, we obtained figures that can be compared with the same sample of air-dried wheat previous to its extreme desiccation (Table I).

The increased speed of germination of the drier sample can be seen, while it will be noticed that the higher speed of germination took place in the presence of appreciably less total water in the case of the drier sample which, however, in the next 30 hours of germination took up

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TABLE I

AIR-DRIED AND EXTREMELY DESICCATED WHEAT WITH PERCENTAGE GERMINATION AND AMOUNT OF WATER ABSORBED DURING GERMINATION

Moisture in original wheat	Moisture, per cent of original wheat absorbed up to germination in 51 hours at 30° C.	Moisture, per cent of bone-dry wheat solids absorbed up to germination in 51 hours at 30° C.	Percentage germination at 30° C. in 51 hours	Moisture, per cent of bone-dry solids absorbed up to germination in 81 hours at 30° C.	Percentage germination at 30° C. in 81 hours
P. ct.					
14.30 (air-dried)	23.86	44.53	5	47.72	11
0.66 (dried over calcium chloride)	25.51	26.35	26	60.35	33

much more water than did the air-dried sample. Duvel (1904), on the contrary, working with seeds of the bean, cabbage, lettuce and onion, observed a shrinkage of the seed-coat with loss of moisture, during drying at 30° to 32° C., and again at 36° to 37° C., for 30 days, and a corresponding retardation of germination that led that writer to conclude, when discussing retarded germination in the case of old seeds, that "the older seeds would need time to regain a portion of their natural moisture lost by keeping before absorbing the further moisture necessary for germination." In the case of wheat, however, we have shown an increased speed of germination generally for the drier seeds, and, in some cases, for very old and dry wheat also.

In Table I it will be seen that the same wheat at the two extremes of moisture-content (0.66% and 14.3%), germinates after absorbing about the same amount of water in 51 hours at 30° C., but that a larger percentage germinates in that time in the case of the drier sample, and that the actual moisture present in the seeds during that time of germination is appreciably less in the originally drier sample. In the case of different kinds of wheat, such as English and Indian, differences in speed of germination that have been noticed might arise from a number of factors other than moisture-content; yet we have observed in these cases also, when the difference in moisture-content is great, that the drier samples show greater speed of germination.

It will be seen among other figures in Table II that it is not possible always to state, in the case of old wheats, that a high speed of germination will take place in very dry seeds. On the other hand, the difference in speed of germination between air-dried seeds 16½ years old, con-

taining 16% of moisture (germination 0) and those dried to 0.66% over calcium chloride and of the same age (germination 82% in 120 hours, 3% in 24 hours) is infinite, while the same wheat with 0.66% moisture germinated 100% in 24 hours when 2 years old, as against 95% germination for the fresh wheat with 16% of moisture.

TABLE II

INFLUENCE OF AGE AND MOISTURE-CONTENT OF WHEAT ON SPEED OF GERMINATION AND VITALITY

Sample	Moisture	Germination at 30° C.	Age
	P. ct.	*P. ct.*	
A	16.00	95	Fresh from harvest
	0.66	100 (24 hours)	2 years (in desiccator)
	0.66	82 (120 hours)	16½ years (2 years in desiccator + 14½ years in sealed tube in dark)
		3 (24 hours)	
	16.00	0	16½ years (air-dried and stored in both sealed and open tubes in dark and light)
B	13.20	92	Fresh from harvest
	5.31	92	4 months (in desiccator)
	4.10	91	11 months (in desiccator)
	4.38	83	13½ years (11 months in desiccator + 12 years 7 months in sealed tube in dark)
	1.97	62	10 years (in desiccator)
	4.41	84 (13 days)	16½ years (11 months in desiccator + 15 years 7 months in sealed tube in dark)
	4.38	83 (7 days)	19 years (11 months in desiccator + 18 years 1 month in sealed tube in dark)

The progress of germination at 20° to 23° C. of 300 seeds of wheat that had been dried at room temperatures in a desiccator for 11 months over calcium chloride (moisture 4.10%), then transferred to a glass tube, sealed and stored in the dark for a further 12½ years (moisture 4.6%) is shown below. The following results from these experimental wheats have kindly been reported by Dr. Winifred Brenchley from Rothamsted Experimental Station.

Progress of Germination of Desiccated Wheat 13½ Years Old

2nd day. 84 chitted.
3rd day. 206 chitted.
4th day. 241 germinated, of which 145 showed plumules and 96 radicles. Very slight growth of mould.
5th day. Total of 245 germinated.
6th day. All showing plumules.
7th day. Roots beginning to break through freely.
8th day. Total of 246 germinated.
9th day. Total of 259 germinated. Roots and plumules growing well and seedlings were healthier in appearance than those in experiment 14 (air-dried wheat sealed in tube over calcium chloride for 14½ years in the dark which gave final germination of 12%). Slight mould in

places but caused by infection spreading from the adjacent experiment 16.

10th day. Shoots growing very rapidly, several over 5 cm. long. Total of 266 germinated.

11th day. Total of 268 germinated.

12th day. All non-germinated grains very mouldy and no further chitting had occurred, so experiments were closed. Germination in this case 89%.

It has been observed that wheat kept under dry conditions of storage will often be found with broken seedcoats usually at the apex of that end of the seed that contains the embryo. Irregularity of absorption figures are often due to this cause which allows a rush of water to enter through the open gap instead of a steady flow through the seed-coat which normally acts as a brake. Ruptured seed-coats are often found under normal conditions when slow drying takes place, but are usual in excessively dried seeds.

By the use of a dilute solution of iodine the course of the water absorbed was readily traced in whole wheat seeds. After about 30 minutes' immersion in the dilute iodine solution, the first sign of penetration was observed; one or two black dots appeared at the extreme apex of the grain, and the space containing the embryo began to fill with liquid. At the end of two hours, the majority of the seeds had developed a blackening on the exterior of the rounded side opposite the embryo in the form of an arc to about ⅓ the length of the seed

Fig. 1. Showing course of water-absorption by wheat, using a dilute iodine solution as indicator.

(Figure 1). The reverse side of the seed was only slightly and locally darkened.

On cutting sections of the seeds, it was seen that the iodine solution had penetrated through the membrane separating the embryo from the endosperm the more deeply the nearer to the rounded side of the seed. Sections cut below the black arc were perfectly white showing that the iodine solution had not penetrated the seed-coat surrounding the endosperm.

The direction of the absorption studied by this method is identical

with the direction of removal of starch and protein matter for nourishment of the growing embryo during germination as shown by Whymper (1909) and further illustrated with photographs in "Knowledge" (1913).

Conclusions

Fresh wheat shows an increased speed of germination *at first* in the presence of less water in the wheat berry itself, including that both originally present and absorbed, during germination if it has been highly desiccated, though the amount of total water taken up by these germinating seeds increases rapidly over that of the normal air-dried seeds without showing any corresponding increase in speed of germination.

It seems generally true from our results that the reduction of moisture much below 5% by artificial drying in the cold over calcium chloride, while stimulating the speed of germination considerably in the case of fresh wheat, is less effective than drying down to about 5% of moisture if the wheat is to be stored for some years and still retain its vitality. From our results, it is probable that this is not true for wheat stored in the dark, since it would appear that storage in a desiccator in the light for a long period of time is more destructive to vitality than if the wheat had been dried for some months in a desiccator in the light, then transferred to a tube, sealed, and stored for the remainder of that time in the dark.

Extremely desiccated wheat, as will be shown in a later paper, is very resistant to the destructive action of heat.

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THE AMYLASES OF NORMAL AND GERMINATED WHEAT FLOURS

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(Presented at the Annual Meeting, June, 1934)

It has long been known that the germination of normal grains is accompanied by a remarkable change in the action of their extracts upon starch. Whereas the chief function of the starch splitting enzymes in the resting grain is one of saccharification, an additional property is imparted by germination. This is commonly referred to as liquefaction because of the ability to rapidly convert thick starch pastes into thin watery liquids. In spite of many attempts to reveal the sequence of events taking place during this liquefaction process, its nature remains unknown. It seems probable that a more fundamental knowledge of the structure of the starch granule must be obtained before the process of liquefaction is thoroughly understood.

In addition to the liquefying phenomenon, another characteristic also develops by germination; this has been termed dextrination because it appears that starch is converted into products which have lower reducing powers than maltose and give red or colorless reactions with iodine. This is in contrast to the normal grain where the products still exhibit a blue starch-iodine color even after prolonged reaction. Whether liquefaction and dextrination are separate and distinct processes is not definitely known.

Kuhn (1925) studied the starch-splitting enzymes derived from various sources. Though all converted starch to maltose, some of them apparently yielded the alpha-glucoside while others gave rise to the beta modification. The first was termed alpha-amylase and the second beta-amylase. This finding has created a new impetus in the study of the enzymatic degradation of starch and within the last few years a number of interesting papers have appeared in the literature. Although many of these have been devoted to the barley grains, evidence that a similar condition prevails in wheat is rapidly accumulating. As a result it seems highly probable that sound, normal wheat contains only active beta-amylase. During germination alpha-amylase activity develops and the magnitude of this activity depends upon the technique of the malting process.

In studying the action of these two amylases it becomes desirable to obtain individual preparations of each. For this purpose there are three fairly well defined methods. The first of these is due to Ohlsson (1926, see also Nordh and Ohlsson, 1932) and depends upon the thermolability of the beta-amylase and the acid-lability of the alpha-amylase. Extracts of germinated grain having a pH of 6 to 7 are heated to 70° C. for 15 minutes. This results in the destruction of most of the beta-enzyme. Another extract is cooled to zero degrees and brought to a pH of 3.3 by adding hydrochloric acid. In this way a major part of the alpha-enzyme is inactivated.

We have applied this method to extracts of germinated wheat flours using the Wohlgemuth iodine method for measuring alpha-amylase activity and following the saccharifying power by determining the maltose produced from soluble starch solutions.

TABLE I

PERCENTAGE OF ORIGINAL ACTIVITY POSSESSED BY GERMINATED WHEAT EXTRACTS TREATED ACCORDING TO OHLSSON'S METHOD

	Acidified	Heated
Alpha-amylase	6%	80%
Beta-amylase	91%	12%

A second method was devised by van Klinkenberg (1932) and involves a fractional precipitation with alcohol. If sufficient alcohol to give a 60% concentration is added to a malt extract, the alpha-amylase is precipitated. Addition of further quantities of alcohol to bring the concentration to 80% precipitates a mixture of alpha and beta-amylase. By refractionation quite pure preparations of each amylase can be obtained as dry powders. Van Klinkenberg found that normal wheat was one of the best sources of the beta-amylase because of its abundance and the almost complete absence of the alpha enzyme.

The third method is that of Samec and Waldschmidt-Leitz (1931). At a pH of 3.8 a special form of alumina "$C\gamma$" adsorbed the beta-amylase completely leaving the alpha-enzyme in solution. At a pH of 5.5 both enzymes were removed. These enzymes could then be separated from the alumina by washing with phosphate or citrate solutions.

Studying such preparations as these has revealed several properties characteristic of each enzyme. The maximum activity of the alpha-amylase is at an appreciably higher pH than the beta-amylase; the former occurs at a pH of 5.6 to 5.8 and the latter at 4.5 to 5.1. The alpha-enzyme diffuses more slowly than the beta-enzyme. This property was utilized by van Klinkenberg in testing the purity of his prep-

arations and served as a rather convenient test. A starch-gelatine surface was innoculated with a drop of the enzyme extract and kept cold for several days. Upon staining with iodine a colorless spot resulted if only alpha-amylase was present. A purple ring surrounding the colorless center indicated the presence of both enzymes in the extract.

With regard to maltose production a distinct difference also exists.

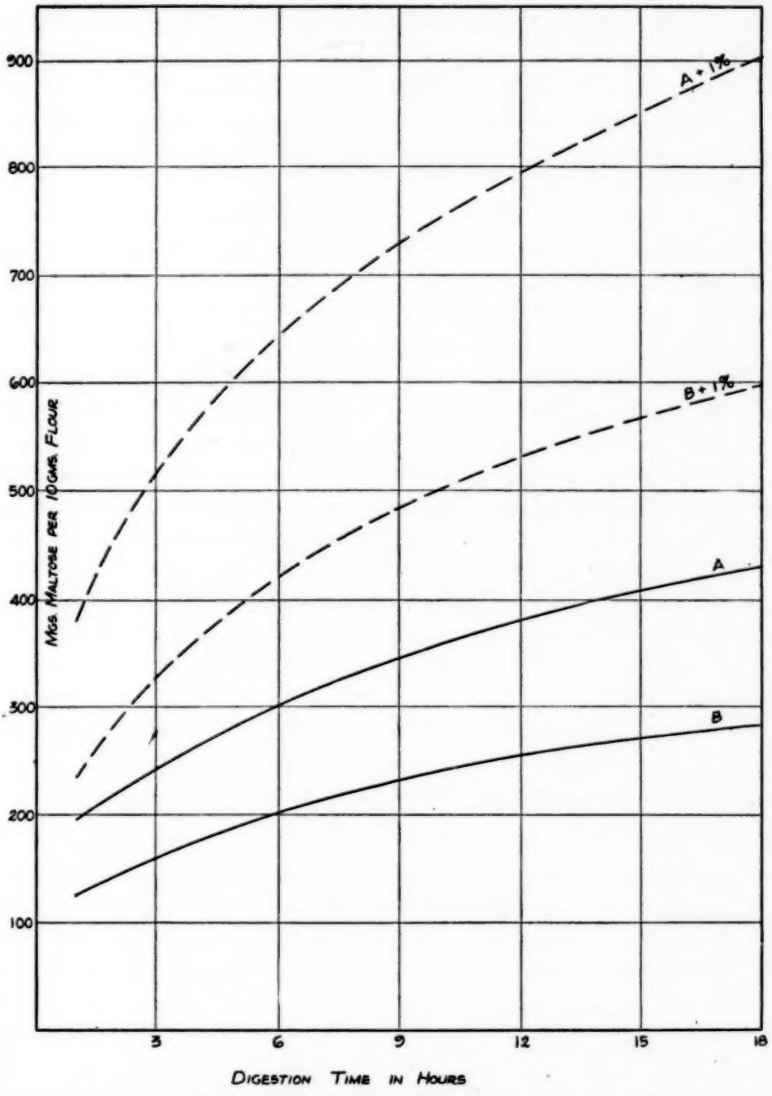

Fig. 1. Effect of added germinated wheat flour upon maltose production of normal flours.
Solid line, normal flours.
Broken line, normal flours containing 1% added germinated wheat flour.

With beta-amylase, maltose production proceeds at a considerably more rapid rate. At the same time a greater quantity of starch is converted to maltose. Because of this fact we have followed maltose production by diastasis of flours at several time intervals in considering the influence of added germinated wheat flours upon diastatic activity.

In Figure 1 are represented two flours—A and B, each having appreciably different maltose-producing abilities. To portions of each flour various quantities (0.2, 1.0, and 5%) of germinated wheat flour was added and maltose production by diastasis in buffered suspensions was measured after various periods of time. Typical responses are indicated by the dotted lines which in this case represent flours to which 1% of germinated material was added. The solid lines represent the original flours.

Measured in terms of actual amounts of maltose produced, flour A exhibits a much greater response. After one hour the increase corresponds to 185 mgs. of reducing sugar. With flour B the increase is only 110 mgs. In order to effect an increase in B equal to that of A it is necessary to add approximately 4% of the germinated material.

From the viewpoint of the amylases involved it would appear that these increases in maltose producing power are due to the alpha-amylase since the amount of beta-enzyme contained in the added material is small in relation to that contained in the original flour. Whether this alpha-amylase produces maltose directly or causes alterations in the starchy substrate so as to bring about a greater saccharification by the beta-amylase is difficult to decide.

In studying these data the results seem more significant if we compare the percentage increase in maltose formation on the basis of the original sugar-producing power of the normal flours. This is represented in Table II.

With both flours the addition of the germinated material resulted in approximately the same relative increase. Although somewhat lower at the end of the first hour the increases are proportional throughout.

TABLE II

PERCENTAGE INCREASE IN MALTOSE PRODUCTION EFFECTED BY ADDING 1% GERMINATED WHEAT FLOUR

Time in hours	Flour	
	A	B
	P.ct.	*P.ct.*
1	95	89
3	112	106
5	112	105
18	111	109

This proportionality suggested that the problem of diastatic activity was more dependent upon the condition of the starchy substrate than upon actual amylase content. A substantiation of this thought was found in the comparison of the action of the flour enzymes of several different flours when some other substrate than the flours themselves were employed.

In Table III are shown the amounts of maltose produced when the flours are autolyzed and when their extracts act upon a solution of soluble starch.

TABLE III

COMPARISON OF MALTOSE PRODUCTIONS BY AUTOLYSIS OF FLOUR SUSPENSIONS AND FLOUR EXTRACTS ON SOLUBLE STARCH (LINTNER)

	Milligrams maltose/10 gms. flour (or extract)	
	Flour itself	Soluble starch
A	207	7300
B	140	7200
C	105	7500

These data show two things: In the first place they indicate that the actual β-amylase activities of three flours are approximately the same and suggest that the differences noted when the natural flours are compared is due to some peculiar condition of the starchy contents. In the second place it will be noted that with the soluble starch the amount of maltose is enormously increased. Either the natural starch of the flours possesses a peculiar resistance to enzymatic degradation or the amount of starch that can be readily broken down by beta-amylase is relatively small. This problem is being studied at the present time.

Summary

In sound normal wheat diastatic activity is due primarily to the action of beta-amylase. When the wheat is allowed to germinate a second "diastatic factor," termed alpha-amylase, is produced. Both these amylases convert starch to reducing sugars, the alpha-amylase giving rise to alpha-maltose and the beta-amylase yielding the corresponding beta sugar. Attending the activation of the alpha-amylase are the phenomena of dextrination and liquefaction. The relation between these two phenomena and alpha-amylase activity is not definitely known.

Utilizing several different properties of the two amylases, several methods are available for their separation. These have been briefly discussed. Other differences between the two amylass are presented. Thus alpha-amylase diffuses more slowly in a gelatin medium and

apparently hydrolyzes soluble starch more slowly than does the beta-amylase. The pH range of optimum activity is 5.6–5.8 while that of beta-amylase is 4.5–5.1.

The increase in rate of maltose production effected by the addition of unit dosages of germinated wheat flour to suspensions of normal wheat flours was positively correlated with the maltose-producing abilities of the normal flours. This appears to indicate that diastatic activity is more a function of the condition of the starchy substrate in the flours than of the actual content of the amylases. Substantiation of this idea was found in the fact that water extracts of several flours possessing widely different diastatic activities yielded nearly equal quantities of maltose when acting upon a soluble starch substrate.

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A STUDY OF CAROTENE CONTENT AND OTHER QUALITY CHARACTERS IN A SERIES OF HYBRID WHEATS [1]

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(Read at the Annual Meeting, June, 1934)

Introduction

It is the endeavour of the wheat breeder to combine desirable agronomic characteristics with high baking strength and with low pigment content of the flour. The creamy or creamy-yellow colour of wheat bread is principally due to the presence of carotenoid pigments, the principal one of which is believed to be carotene itself; consequently a knowledge of the carotene content of a flour is extremely important in predicting the colour of the bread baked therefrom.

Accurate determinations of the carotene content of flour may be carried out by the spectrophotometric method of Ferrari and Bailey (1929), or the more rapid colorimetric method of Geddes, Binnington, and Whiteside (1934).

For the plant breeder who wishes to produce new varieties of wheat yielding flour of low carotene content, these advances in the study of the quantitative determination of carotenoid pigmentation are extremely valuable, but the methods now available are still unsuitable for application to plant breeding work in the early generations. In the F_3 and subsequent generations, until the new varieties are being increased for plot tests, the quantity of seed available of each family is 100 to 300 grams. This means that it is impossible to produce flour from the seed of F_3 families in the ordinary experimental mill. It is in the early generations that carotene determinations should be made as the families of each cross are being handled in large numbers. When they have been advanced to the plot testing stage the numbers have been very greatly cut down and the chances of obtaining strains of low carotene content are very much reduced. To depend thus on the chance selection of low carotene strains can be shown to be a very untrustworthy

[1] Published as paper No. 57 of the Associate Committee on Grain Research, National Research Council of Canada, and as paper No. 10, Dominion Grain Research Laboratory, Board of Grain Commissioners, Winnipeg.

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method. In the group of 48 strains of an H–44–24 × Marquis cross, for which data are given in this paper, less than 10% were equal or superior to Marquis, considering the quality characters, crumb colour of the bread, and flour strength. From a quality standpoint the breeding methods used were therefore only 10% efficient.

In order to obviate these difficulties and enable the plant breeder to obtain information on wheat samples too small for milling into flour, Whiteside, Binnington, and Geddes (1933) attempted to relate the carotene content of ground whole wheat to that of a straight grade flour milled therefrom. The carotenoid content of bran, germ, and endosperm differs widely, the highest concentration being found in the germ, and as the relative proportions of these constituents vary in different samples, it is obviously difficult to obtain results on the wheat that can be directly related to the flour. Furthermore, in comparing varieties, there is the probable additional factor of variety differentiation with respect to the proportionate amounts of carotene in the various parts of the wheat kernel. Nevertheless it seems feasible that for plant breeding purposes successive elimination through two or three generations of all strains showing a high carotene content of the whole wheat will result finally in the selection of strains that are low in carotene content of the flour.

The present study was undertaken with a view to an exact determination in a group of new varieties of the relation between the carotene content of the flour and that of the whole wheat. After such a study it was felt that more definite experiments could be planned for the determination in wheat breeding studies of the efficacy of the method of eliminating hybrid material on the basis of carotene content of the whole wheat.

Materials

The wheats studied were made up of groups of hybrid strains and a group of standard varieties as indicated in Table I.

TABLE I

DESCRIPTION AND NUMBERS OF WHEAT STRAINS STUDIED FOR CAROTENE CONTENT AND OTHER QUALITY CHARACTERISTICS

Group	Number
Standards	11
Miscellaneous Hybrids	12
H-44-24 × Marquis Hybrids	48
H-44-24 × Reward Hybrids	30
Pentad × Marquis Hybrids	31
Double Cross × Ceres Hybrids	3
Double Cross × Marquis Hybrids	4
Total	139

Methods

The wheats were grown at Winnipeg in 1933. Small portions of each sample were retained for protein and carotene studies in the Dominion Grain Research Laboratory and the balance forwarded to the Cereal Division, Ottawa, for the determinations of weight per 1000 kernels, test weight per bushel, and experimental milling and baking tests. The samples were milled to straight grade flours and baked by a 100-gram formula containing 0.001% $KBrO_3$, 0.3% diastatic malt (approximately 200° Lintner) and 0.1% $NH_4H_2PO_4$ in addition to the ingredients specified in the A. A. C. C. basic baking tests. The conditions laid down in the A. A. C. C. procedure were followed with the exception that the absorption was varied to suit the flour and baked in "low form" tins, the doughs being mixed in a Hobart-Swanson mixer for one minute.

Carotene determinations were run on extracts prepared from both the straight grade flours and wheats, the latter being prepared by grinding in a Wiley mill fitted with a screen perforated with round holes 0.5 mm. in diameter. Two solvents were employed in each case. For the flours the solvents were varnish makers' naphtha, as described by Geddes *et al.* (1934), and the same solvent containing 7% by volume of absolute ethyl alcohol. In the instance of the ground wheats, the naphtha and naphtha-alcohol solvents were also employed, but the alcohol content was increased to 10% as Whiteside *et al.* (1933) found that this increase was necessary to secure maximum extraction. In all cases, 20-gram samples were extracted with 100 cc. of solvent and the transmittances of the extracts determined at 4358 Angström according to the method of Shertz (1923), using a Bausch and Lomb spectrophotometer.

Statistical Studies

Statistical studies were made on the data for carotene in the flour (f), carotene in the whole wheat, solvent naphtha (n), carotene in the whole wheat, solvent naphtha plus alcohol (a), weight of 1000 kernels (k), weight per bushel (w), loaf volume (l), flour yield (y), and per cent protein (p).

Correlation coefficients for the entire series of wheats were determined for all possible pairs of variables. These are given in Table II. Some of these coefficients are of considerable interest. The relation between carotene in the flour with that in the whole wheat is indicated by $r_{fn} = .7024$, $r_{fa} = .6584$, $r_{na} = .7225$. The slightly higher correlation between carotene in the flour with carotene in the whole wheat using the naphtha solvent, as compared with that using naphtha plus alcohol, is due largely to the higher variability of the latter method.

TABLE II

CORRELATIONS FOR NINE QUALITY FACTORS IN A MISCELLANEOUS GROUP OF 139 STRAINS AND VARIETIES OF WHEAT

Carotene			Weight of 1000 kernels	Weight per bushel	Loaf volume in cc.	Crumb colour score	Yield of flour	Protein in flour
Flour (*f*)	Wheat (*n*)	Wheat (*a*)	(*k*)	(*w*)	(*l*)	(*c*)	(*y*)	(*p*)
f	.7024	.6584	−.2199	−.1992	.0766	−.5791	.0151	−.0278
n		.7225	−.1558	−.2704	.1340	−.5216	.0052	.0572
a			−.1532	−.1714	.1074	−.4951	.0030	−.0443
k				−.0806	.1455	.2023	.0797	.1104
w					−.0928	.0794	−.1615	−.3217
l						.4797	.0724	.6681
c							.1037	.3531
y								.0471
p								

(*n*) Solvent naphtha.
(*a*) " " plus 7% alcohol.

Figure 1 showing the carotene results for the H–44–24 × Reward group in the same order as the plots were placed in the field gives some clue to this effect. The carotene results by naphtha plus alcohol show more tendency to vary with changes in the soil than the other two measures of carotene. This difficulty might have been overcome by replica-

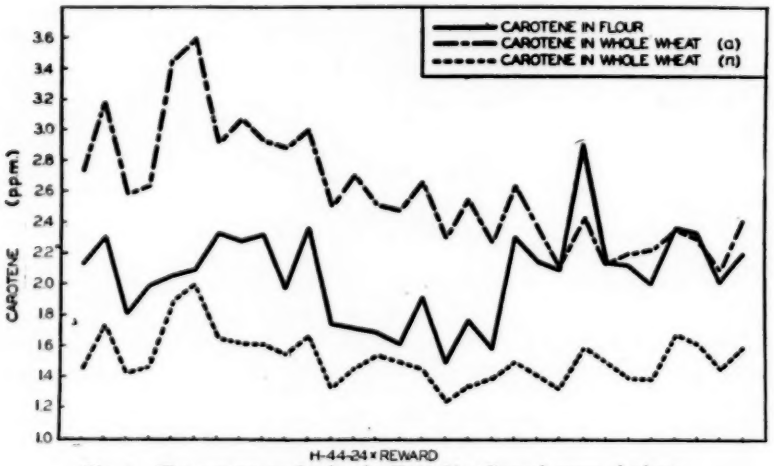

Fig. 1. The carotene results for the H–44–24 × Reward group of wheats.

tion but the necessity for this was not realized at the time the plantings were made.

The second group of correlation coefficients of interest are those for carotene content with the crumb colour score. These are $r_{fc} = -.5791$, $r_{nc} = -.5216$, and $r_{ac} = -.4951$. Here again there is some indica-

tion that the naphtha solvent method is slightly better for determinations on whole wheat than the naphtha plus alcohol. These correlations are not as high as might be expected if carotene is the chief contributor to the colour of the bread but of course texture must have some effect on the colour as it appears to the eye and the decided effect of volume on the distribution of the carotene is evident from the coefficients $r_{lc} = .4797$, $r_{pl} = .6681$, and $r_{cp} = .3531$. These merely reflect the fact that with stronger flours a given quantity of carotene is distributed throughout a greater volume and a lighter colour results.

The relation between carotene content and weight per bushel and weight of 1000 kernels is of interest as it might be expected that these measures of kernel density and size would bear a closer relation to carotene in the whole wheat than to carotene in the flour. From a study of the total correlation coefficients there is evidently very little of such an effect in this series. It should be emphasized at this point, however, that the material studied is a series of genetically different varieties and not a series of genetically similar samples grown under different environmental conditions. Environmental factors that change kernel weight and weight per bushel may have a noticeable effect on carotene content but unless there is a direct causal relationship between these characters similar effects would not necessarily be obtained in a study of a series of varieties grown under identical environmental conditions. In a series such as the one studied there is also the question of genetic linkage to be considered in that in certain crosses groups of factors tend to remain together. The true effect of weight per bushel on crumb colour is indicated by the results from the partial correlation

TABLE III

SELECTED TOTAL AND PARTIAL CORRELATION COEFFICIENTS FOR ENTIRE SERIES

	Total	Partial		Total	Partial
cf	−.5857	−.5791	*cn*	−.5216	−.6664
cp	.4232	.3531	*cl*	.4797	.5408
cw	.1085	.0794	*cw*	.0794	−.0408
wf	−.1127	.1992	*nl*	.1340	.5955
wp	−.3468	−.3217	*nw*	−.2704	−.2267
fp	.1848	−.0278	*lw*	−.0928	−.0140
5% pt.	.1684	.1696		.1684	.1696

studies as given in Table III. Considering the group on the right in which the characters studied are, crumb colour (c), carotene (n), weight per bushel (w), and loaf volume (l), the partials of (c) with the other characters are $r_{cn} = -.6664$, $r_{cl} = .5408$, $r_{cw} = -.0408$. The last

coefficient is quite insignificant therefore in the multiple regression equation

$$R^2_{c.nlw} = (B_{cn}.r_{cn}) + (B_{cl}.r_{cl}) + (B_{cw}.r_{cw});$$

the last term is obviously insignificant and does not add significantly to the value of the multiple correlation coefficient. In other words, from the standpoint of predicting crumb colour, when the carotene in the whole wheat and the flour strength are known, the weight per bushel is of no value. Similar results, as given on the left in Table III, are obtained using the characters crumb colour, carotene in the flour, protein, and weight per bushel. The partial correlation $r_{cw.pf}$ is quite insignificant.

From the standpoint of a study in plant breeding it is more useful to consider a group of strains from one cross. Consequently the results from the largest group—the 48 strains from the cross H–44–24 × Marquis—were subjected to the same type of analysis as given above for the entire series (Figure 2). The total correlation coefficients are given in Table IV. These are somewhat similar to those for the entire series

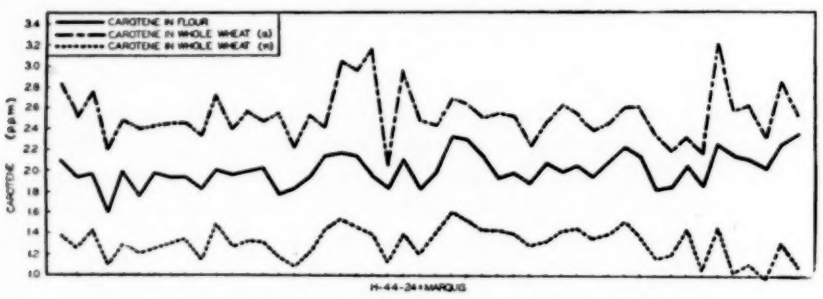

Fig. 2. The carotene results for the H–44–24 × Marquis group of wheats.

TABLE IV

CORRELATIONS FOR EIGHT QUALITY FACTORS IN A GROUP OF 48 STRAINS OF THE CROSS H-44-24 × MARQUIS

Carotene		Weight of 1000 kernels	Weight per bushel	Loaf volume in cc.	Crumb colour score	Yield of flour	Protein in flour
Flour (*f*)	Whole wheat (*n*)	(*k*)	(*w*)	(*l*)	(*c*)	(*y*)	(*p*)
f	.5275	−.1510	−.0805	−.2816	−.4664	−.0065	−.4692
n		−.2725	−.3845	−.1280	−.4020	−.2745	−.2016
k			.0241	.0812	.0847	.2189	.1242
w				−.2444	−.0091	.1227	−.2601
l					.7494	.3076	.7190
c						.3635	.5312
y							.1197
p							

(*n*) Solvent naphtha.

with a few important exceptions. In the first place we have the negative coefficient $r_{fp} = -.4692$ showing a marked relationship between protein and carotene. This looks like a genetic relationship but is difficult to understand considering the results on protein and carotene for the two parents as given in Table V. Judging from previous results as

TABLE V

CAROTENE AND PROTEIN DATA FOR PARENTS H-44-24, AND MARQUIS

	Protein	Carotene	
		(*f*)	(*a*)
H-44-24	15.1	2.02	2.57
Marquis	14.3	1.76	2.35

well as these the tendency has been for H–44–24 to possess a slightly higher carotene content than Marquis and about the same percentage of protein. In the second place we have the significant coefficients $r_{cy} = .3635$ and $r_{ly} = .3076$. The situation bringing about this result is probably complex and the coefficients are too small to justify a much more detailed analysis. It would seem that the attribute of giving a high flour yield due to size of kernel and ease of separation of the bran results in a purer flour and consequently a better colour. This may result indirectly, however, from the higher loaf volume given by the finer grade of flour. A third point is that carotene in the wheat seems to be affected to a somewhat greater extent by kernel characteristics than carotene in the flour. This is evident from the coefficients $r_{fk} = -.1510$, $r_{nk} = -.2725$, $r_{fw} = -.0805$, and $r_{nw} = -.3845$.

There are two phases of this study of the data from the H–44–24 × Marquis hybrids that are of interest to the plant breeder. The first is the relationship of carotene in the wheat and other quality characteristics to carotene in the flour, and the second is the relationship of all of these factors to crumb colour. The analysis of the data was directed therefore first towards the selection of those factors that could be determined on a small sample of whole wheat and used to estimate the carotene content of the flour. The total and partial correlation coefficients concerned are given in Table VI.

Those partials with *f* as one of the variables that are significant are italicized. There are only two, $r_{nf.kwp} = .4461$ and $r_{pf.nkw} = -.4131$. We conclude therefore that in this cross the only two factors of value in estimating carotene in the flour are carotene in the wheat and per cent protein. Calculating the multiple correlation coefficient as in Table VII we find $R_{f.np} = .6446$ and this represents a significant gain in informa-

TABLE VI

SELECTED TOTAL AND PARTIAL CORRELATION COEFFICIENTS FOR 48 H-44-24 × MARQUIS Strains

	Total	Partial		Total	Partial
nk	−.2725	−.2411	*nk*	−.2725	−.2669
nw	−.3845	−.4155	*nw*	−.3845	−.4414
np	−.2016	−.0731	*np*	−.2016	−.1054
nf	.5275	*.4461*	*nc*	−.4020	*−.3302*
kw	.0241	−.0684	*cp*	.5312	*.4751*
kp	.1242	.0495	*cw*	−.0091	−.0130
kf	−.1510	.0253	*ck*	.0847	−.0762
wp	−.2601	−.3390	*wp*	−.2601	−.3186
wf	−.0805	−.0065	*wk*	.0241	−.0693
pf	−.4692	*−.4131*	*kp*	.1242	.0738
5% pt.	.2828	.2886		.2828	.2886

tion for estimating flour carotene over using either one of the total correlation coefficients.

Turning to the study of crumb colour we have the total and partial correlation coefficients necessary on the right of Table VI. The significant partials with crumb colour as one of the variables are $r_{nc} = -.3302$ and $r_{pc} = .4751$. The multiple correlation coefficient $R_{c.np} = .6106$ and again there is a significant gain in precision in the estimation of crumb colour over the separate use of either carotene in the wheat or per cent protein.

Two other multiple correlations were calculated that are of some interest. The first is $R_{c.fp} = .5853$ which compares with $R_{c.np} = .6106$. While the difference between them is not significant it is remarkable that the latter is the larger of the two. In other words, there seems to be no evidence, considering the two variables carotene and protein for the estimation of crumb colour, that the carotene content of the flour is any more valuable than that of the whole wheat.

The fourth multiple correlation $R_{c.lp} = .7495$ is almost identical with $r_{cl} = .7494$. This is as would be expected in that the value of protein for estimating crumb colour is due mainly to its reflection of the strength of the flour.

Discussion

In a study of this kind, giving results for only one year and in detail on only one cross, one is not justified in coming to any definite conclusions regarding the use of carotene determinations on whole wheat in the breeding procedure. If both of the correlations r_{cf} and r_{nf} had been higher the evidence would have been much more satisfactory, but possibly in a series of genetically different wheats, such as the one studied, higher correlations may rarely be obtained. The results seem to indi-

TABLE VII

CORRELATION ANALYSIS FOR CRUMB COLOUR, CAROTENE IN FLOUR AND WHEAT, PROTEIN, AND LOAF VOLUME FOR 48 STRAINS OF THE CROSS H-44-24 × MARQUIS

	Total	Partial	Multiple
cf	−.4664	−.2903	
cp	.5312	.3999	$R_{c.fp}$ = .5853
fp	−.4692	−.2954	
cn	−.4020	−.3554	
cp	.5312	.5020	$R_{c.np}$ = .6106
np	−.2016	.0153	
cp	.5312	.0165	
cl	.7494	.6242	$R_{c.lp}$ = .7495
lp	.7190	.5721	
fn	.5275	.5004	
fp	−.4692	−.4361	$R_{f.np}$ = .6446
np	−.2016	.0612	

cate, however, that there are possibilities in the whole wheat determinations. Using the colorimetric method given by Geddes *et al.* (1934) carotene determinations can be made on whole wheat samples on a large scale and if these are first made on the wheat from the F_3 families they can be repeated on the progenies of the same families for at least two more years. If all families giving high carotene results are discarded there is certain to be a marked improvement in the carotene content of the flour of the strains finally produced.

In certain crosses, such as the H–44–24 × Marquis, unexpected relations may occur such as the negative correlation between carotene and protein. It will require further study to determine how information of this sort may be utilized.

Summary

A series of 139 miscellaneous and new hybrid strains of wheat were grown in 1933. The wheat was milled and baking tests made, and carotene determinations were carried out on the flour and the whole wheat.

A statistical study of the relation between carotene in the flour and carotene in the whole wheat indicated that tests for carotene in the whole wheat might be of considerable value in breeding work if applied in the early generations and continued on the progeny for two or three years.

The series of wheats studied contained one group of 48 strains from an H–44–24 × Marquis cross. In this group the results were similar to those for the entire series except for a negative correlation between carotene and protein.

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ALUMINUM DESICCATOR PLATES

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(Presented at the Annual Meeting, June, 1934)

The purpose of quick ash and rapid moisture tests is defeated if much time is lost in cooling the ash capsules and moisture dishes after they are taken from the muffle or moisture oven. Rapid cooling is impossible on ordinary porcelain desiccator plates.

Cooling time is materially reduced when capsules and dishes are placed on an aluminum disc in the desiccator. A pair of discs will be an advantage for a long series of tests. Silica ash capsules may be handled for direct weighing in three to five minutes, and for indirect weighing in about ten. Large moisture dishes when handled one at a time, as in the case of the rapid oven, may be weighed back in from one to three minutes. Dishes from the vacuum or air oven, when handled six or eight at a time, may be safely weighed back in from five to ten minutes. The efficiency of the cooling will depend upon the size of the discs used and upon the number of test dishes being handled. In any event the rate of cooling will be several times as fast as on porcelain. The discs may be cooled under the tap and dried satisfactorily with a towel. If covered moisture dishes are used, even a desiccator may be found unnecessary in dry climates for a chilling period of less than five minutes.

Caste aluminum discs of sizes suitable to fit the desiccator in question may be obtained from the metal foundry. A disc five and one-half inches in diameter by one-half inch thick, highly polished on one surface and the edges, costs about $1.50.

THE DETERMINATION OF THE WATER ABSORBING CAPACITY OF FLOUR BY MEANS OF THE SUPER CENTRIFUGE [1]

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(Presented at the Annual Meeting, June, 1934)

The absorption capacity of a flour is frequently discussed as though it were a definite value, such as protein content. This is readily seen to be erroneous, since the correct absorption varies with baking method, fermentation time, and severity of mixing. Nor can the amount of variation in absorption for any such change be accurately estimated—the absorption of a strong flour increases greatly with high speed mixing, that of a weak flour often does not increase, and may easily be decreased by overmixing. The correct absorption for a flour high in enzymes decreases rapidly with increase in fermentation time; that for a flour low in enzymes decreases only slowly. Further, changes in the absorption used for a given flour change certain characteristics of the resulting bread, but the selection of one type of bread as best is only arbitrary, since preferences vary.

The absorption of a flour is often defined as the percentage of water necessary to give a dough of the correct consistency after mixing. This is open to criticism, since the amount of mixing is not specified, and especially since the consistency at the time of molding the loaf is much more important, also the consistency from this time up to the time the structure of the loaf is set by the oven heat. But this is not the only difficulty in determining absorption by instruments designed to measure dough consistency, for flours which differ widely in protein quality or quantity must necessarily be mixed to different consistency if they are to produce the best bread of which they are capable.

If flour is suspended in water, allowed to digest thirty minutes, and then most of the water removed by means of the super centrifuge, an accurately reproducible measurement of the water absorbing capacity of the flour under these conditions can be made. With careful selection and control of the details of the treatment, this method has given a very much more accurate indication of the correct amount of water to use for experimental baking than any other method which has been tried in this laboratory.

[1] Subcommittee report, 1933–34 Committee on the Standardization of Laboratory Baking.

The baking conditions used include considerable mechanical gluten development, a rather long straight dough fermentation, and the use of tap water for both baking and absorption determination. If only a minimum of mixing were used in baking, the absorption indicated by the centrifuge would be too high in the case of many flours of very high protein content. The possibility of using distilled water throughout has not been investigated. It appears that the presence of certain salts in the water used has opposite effects on the absorption as determined by the centrifuge and that most desirable for baking. Variations in fineness of granulation also cause some discrepancy between the absorption indicated by the centrifuge and that desired for baking, but this is usually less than 1% when the soaking period of thirty minutes is used in the determination instead of the five-minute period originally used. This longer soaking time also reduces the error due to unusually high enzyme content in some flours.

Percentage of all flours baked		
Crop of 1932	Crop of 1933	Errors in absorption
70.3%	65.6%	Less than 0.5%
2.4	11.8	.5 to 1.0
22.1	6.4	1 to 1.5
1.2	5.3	1.5 to 2
1.2	5.3	2 to 3
2.5	5.3	Over 3

The absorption shown by the centrifuge is still not considered as final, but provision is made for adding water during mixing of the dough. With the mixer being used, experienced observation indicates clearly when a dough is too dry if the flour type is known. If the dough produced is too slack, the excess water is estimated and if the error appears sufficient to noticeably affect the baking results, another dough is mixed. These corrections are tabulated to indicate the errors of the absorption determination. Of course there are undoubtedly times when the correction made is not sufficient to obtain optimum dough condition, but over-correction is fully as frequent.

The distribution of errors for all the flours baked here during the past two years is shown below. These include commercial flours from all over the United States and Canada, and experimentally milled flours from wheats from our own experimental plots, from collaborating experiment stations, and from foreign countries. By far the greater proportion of the errors shown occur in the testing of new varieties or varieties not suited to bread production, and in those wheats grown under abnormal conditions.

MODIFIED PROOFING CABINET

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(Presented at the Annual Meeting, June 1934)

Since temperature affects baking results so conspicuously, no matter what system is used, reliable control is imperative. And since few may feel able to dispose of imperfect apparatus in favor of the latest the market affords, the following suggested modifications are made for improved temperature and humidity control by relatively simple and inexpensive means, which should be of value to laboratories with equipment which can be modified for efficient use.

The problem in each will involve adequate circulation of air, provision for humidity, temperature control by means of a sturdy, efficient, properly located thermo-regulator, and sufficient insulation.

This laboratory has a two-shelf-type cabinet with ice chamber above connected by ducts from the top of the cabinet and by a return pipe at the back for distributing the cool air into the bottom through a manifold. Convection currents or gravity flow, upon which it originally depended, made hot weather use a virtual impossibility, and automatic control erratic at any time. This cabinet has been modified to give practically perfect control over temperature within a range of not over $\pm 0.5°$ C. together with desirable humidity conditions in either summer or winter on the full surface of the upper shelf (use of the lower shelf was abandoned).

Adequate, but not too severe, circulation of air was achieved only after various fans and blowers had been tried in all conceivable ways. A cheap blower (operating on 110 volts, similar to that used on some automobile heaters, and costing less than $5.00) with housing removed, made an excellent circulating fan. Two of these were used—one, mounted on the lower shelf, distributes the heated air latterally across the heater elements at a rate sufficient to cause a gentle circulation of air within the cabinet and to prevent accumulation of heat on or over these heaters; the other, located in the upper elbow of the return pipe behind the ice chamber, circulates the air over the ice, returning it to the bottom of the cabinet just below the heater elements.

Garrett [1] described a shunt circuit for operating a fan to cool a constant temperature water bath in which the fan drew current through the

[1] Garrett, Alfred B. A cooling device for operating a water thermostat a few degrees below room temperature. Ind. Eng. Chem. 25: 355 (1933).

heaters, when off; but due to the high resistance of the windings, did not operate when the heaters were switched into the circuit by the thermo-regulator.

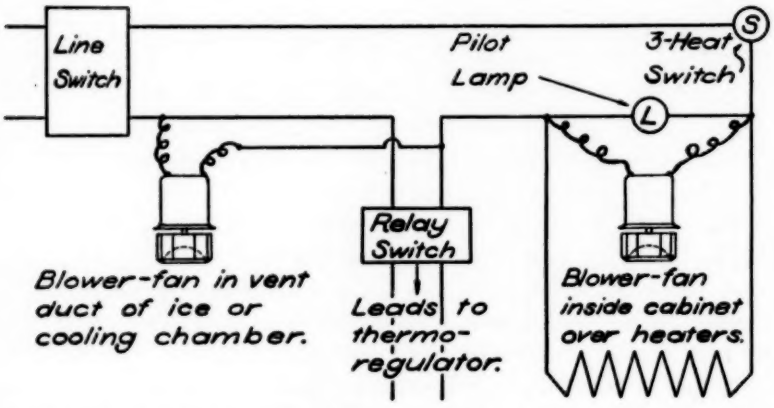

Fig. 1. Diagram showing shunt circuit connections for two fans in modified proofing cabinet. such that only one operates at a time—the one over the heater elements during the heating phase, the one in the ice chamber vent duct during the non-heating or cooling phase.

As shown in Figure 1, this scheme was used in attaching the ice-chamber fan to the circuit so that it operates only when the heaters are "off." The fan on the lower shelf inside the cabinet operates only when the heaters are "on." Constant mechanical circulation of a very desirable type is thereby obtained. No heat is permitted to accumulate over the elements, no excess heat is developed by the motor inside the cabinet, circulation through the cooling chamber takes place only when the heaters are "off" resulting in economies in the amount of ice necessary for controlling temperature in hot weather.

Adequate humidity is provided by dampened wicks of outing flannel hung on frames to fill the space between the upper and lower shelves. Air from the blower over the heaters maintains from 90% to 95% relative humidity. Circulation is baffled sufficiently by the damp cloths so that the dough surfaces are neither dry nor too wet. Air from the ice chamber also is baffled enough so that sufficient moisture is absorbed to maintain the proper humidity even when ice is used for cooling.

False doors of presswood, in front of the wicks, permit exposing only the shelf in use when the doors are opened. A sturdy, dependable thermo-regulator with suitable relays may be obtained for from $20.00 to $30.00. The individual cabinet must be studied to determine the best location for the instrument if sensitive control is to be realized. An auxilliary cooling chamber can be cheaply constructed to obtain the advantages outlined if a cabinet requiring improvement is without the ice chamber and ducts described.